REPORT OF THE
NORTHERN REGIONAL RESEARCH CENTER

December 31, 1976

North Central Region
Agricultural Research Service
UNITED STATES DEPARTMENT OF AGRICULTURE

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PROGRESS REPORT
OF THE
NORTHERN REGIONAL RESEARCH CENTER
December 31, 1976

INTRODUCTION

The Northern Regional Research Center, located at Peoria, Illinois, is one of the major research centers of the North Central Region, Agricultural Research Service, U.S. Department of Agriculture. Basic and applied research is conducted in the physical and biological sciences and in engineering. Northern Center scientists cooperate with representatives of colleges and universities, State experiment stations, research institutes and associations, industrial organizations, and with other Government agencies. Much of the cooperation is informal, but some work is conducted under cooperative agreements and memorandums of understanding. In addition, the Center's program is supplemented by a variety of research projects in foreign countries under Public Law 480 grants.

Providing scientific information for improvements in the post-harvest sector of American agriculture is a major mission of NRRC. Such improvements in the handling, storing, processing, and distribution technology through which agricultural products move "from farm gate to shopping basket" inevitably benefit both consumers and farmers. For example, research on this complex technology provides the basis for preserving and increasing food quantity, economy, quality, safety, and nutritive value. This research also offers our best hope for substantially reducing the energy intensiveness of post-harvest operations, which collectively require at least twice as much energy as is consumed on farms, and for innovations contributing to maintenance of environmental quality. Responding to these needs and opportunities, NRRC's research program and scientific staff provide ARS's principal effort and expertise in processing and utilizing soybeans, cereal feed grains, and special crops. In addition, the Center is one of two in the Agency devoting substantial attention to uses of agricultural byproducts and residues.

Currently most of the soybean research is directed toward decreasing the cost and improving food quality of the oil and of high-protein products derived from the meal. This research continues to justify the stature earned by past accomplishments which provided much of the fundamental basis for today's edible soybean oil and food grade soy protein industries. In the nonfood area, a nucleus still exists of a former sizeable effort to exploit soybean and other vegetable oils as petrochemical-sparing industrial raw materials. From this nucleus, the research could be expanded rapidly, should national priorities so mandate.

Cereal grain research, on the other hand, is almost equally concerned with industrial and with feed and food uses. For example, Center scientists' special knowledge of the complex chemical and physical properties of corn starch and flour has enabled them to develop new technologies with promising on-farm applications. These include water pickup and retention by Super Slurper and controlled release of pesticides through encapsulation with derivitized starch. Recent research in the food area led to corn germ flour, and studies on food fiber are underway. In addition, the Center staff continues to be a principal source of information and research on blended foods, such as CSM (from corn, soybean flour, and dry milk solids) for the Food for Peace program. Another major contribution to food science is research to determine the relationship between wheat proteins and their functional properties in bread and other baked goods. Ultimately these studies will provide fundamental insight relevant to nutritional products from other cereals as well as from oilseeds.

Among several special crops under study, kenaf is of particular interest because of its large yield of dry matter per acre and the possibility of growing it on disturbed lands. Kenaf research at the Center is aimed at providing a supplementary raw material for making paper. This research, like that on agricultural byproducts and residues, also provides a reservoir of information on which future technologies to produce and conserve energy may be based.

Two key research resources--an experimental papermaking machine that is one of the world's most versatile and the ARS Culture Collection--are invaluable tools in the Center's program. Critical for evaluation of novel concepts for new starch-based paper additives, the paper machine is presently used in studies to reduce pollution from papermaking effluents and to produce experimental papers from kenaf. The ARS Culture Collection is a world renowned repository of agriculturally and industrially important microorganisms. Reference cultures, catalogued taxonomic data, and professional expertise associated with this microbial germplasm bank have enabled NRRC to make vital contributions and to assume preeminent roles in mycotoxin research and in fermentation technology, including production of food ingredients and fermented foods. Because of the unique capabilities of a multidisciplinary staff and the importance of the problem, research on mycotoxins has become one of the largest components of the Center's overall effort.

Post-harvest processing behavior and product quality are markedly affected by pre-harvest factors. Recognizing this, Center chemists, engineers, microbiologists, and physicists participate in joint projects with other ARS and SAES scientists conducting genetic and agronomic studies. Determination of processing and compositional characteristics of plant materials from botanical collections, breeding programs, and studies of soil and atmospheric variables is a major form of such

participation. Another involves natural toxicants. Center scientists provide analytical and biochemical information necessary to make sure levels of these minor constituents are not seriously increased in new varieties.

In the newest dimension of the Center's research program its scientists have added their weight to the growing emphasis on photosynthesis, nitrogen fixation, and plant tissue culture. Their novel biochemical, microbiological, and physical approaches complement longer standing studies by plant physiologists and thereby expand and diversify the total ARS attack in these high priority areas.

This report summarizes current research of the Center and lists publications and patents resulting from the research. The research summaries include some tentative results that have not been tested sufficiently to justify general release. Such findings, when adequately confirmed, will be released promptly through established channels. Because of this, the report is not intended for publication and should not be referred to in literature citations. Copies are available to those having a special interest in the development of public agricultural research programs.

This report was prepared at the Northern Regional Research Center, Agricultural Research Service, U.S. Department of Agriculture, Peoria, Illinois 61604. Additional copies of the report and reprints of most publications can be obtained from the Northern Regional Research Center. A separate semiannual listing of publications and patents also is available.

RESEARCH FOR NEW PRODUCTS AND PROCESSES AND FOR REDUCING COSTS

CRIS Work Units Listed by Work Reporting Unit

Work Reporting Unit (WRU)

Number	Title (Principal Investigator)
TECHNOLOGIES FOR FOOD AND FEED USES OF FIELD CROPS (p. 8)	
3102-15650-009	Selective treatments for removal of beany and bitter components from soybeans (J. J. Rackis)
3102-20520-001	Structural and chemical composition of indigenous maize races (F. R. Dintzis)
3102-20520-002	Immobilized enzyme studies for processing cereal grains (K. L. Smiley)
3102-20520-003	Effect of mold enzymes on undesirable soybean factors (H. L. Wang)
3102-20520-004	Germ plasm bank of microorganisms for research in cereal microbiology (T. G. Pridham)
3102-20520-005	Characteristics and classification of *Rhizopus*-like fungi from corn and other cereal grains (J. J. Ellis and C. W. Hesseltine)
3102-20520-006	Investigation of fermentation stage of wild rice processing (H. L. Wang)
3102-20520-007	Effects of isoelectric precipitation and of heat on soybean proteins (W. J. Wolf)
3102-20520-008	Conversion of cellulosic materials to ruminant feed by solid substrate fermentation (R. W. Detroy)
3102-20520-009	Parching process for wild rice (R. A. Anderson)
3102-20520-010	Color development in wild rice (G. E. Inglett)
3102-20520-011	Enzyme modification of feed and cellulosic materials for improved beef animal feed (C. W. Blessin)
3102-20520-012	Conversion of cereals and/or their fractions and components to new or improved foods (R. A. Anderson)
3102-20520-013	Molecular structure of cereal-derived sugars, sugar-derived compounds, and their complexes (J. E. Hodge)
3102-20520-014	*cis*-Bond forming hydrogenation and autoxidation studies to improve edible soybean oil (E. N. Frankel and H. W. Gardner)
3102-20520-015	Functional properties of proteins in wheat and related grains, their flours, and protein isolates (J. S. Wall)

3102-20520-016	Mass spectrometry of deuterium labeled blood lipids, soybean oil odors, and other research samples (W. K. Rohwedder)
3102-20520-017	Continuous hydrogenation of soybean oil for export and domestic markets (C. R. Scholfield and K. J. Moulton)
3102-20520-018	Preparation of protein products from corn and related cereals (J. S. Wall)
3102-20520-019	Rapid characterization of yeasts through genetic and DNA/DNA hybridization and computer analysis (C. P. Kurtzman)
3102-20520-020	Germ plasm bank of microorganisms for research on plant residue utilization (T. G. Pridham, L. K. Nakamura, and A. J. Lyons)
3102-20520-021	Upgrading quality of products from damaged or contaminated soybeans (T. L. Mounts, E. D. Bitner, and G. R. List)
3090-20522-022-A	Separation of immature green wild rice seed from mature green rice (R. A. Anderson)
3102-20520-023	Dietary fibers and residual lipids in defatted soybean protein products (J. J. Rackis)
3102-20520-024	Composition and properties of seed lipids for foods and feeds (J. A. Rothfus)
3090-10571-001-A	Methods for treating and handling green wild rice (R. A. Anderson)
0703-10572-002-A	Treating and storing green wild rice to lengthen processing season (R. A. Anderson)
8002-20520-034	Conversion of cellulosic wastes into feed for ruminants (C. W. Hesseltine)
8003-20520-054	Effect of tocochromanol dimers and trimers on the autoxidation of fats (E. H. Pryde)
8003-20520-064	Thermophilic microbial conversion of cellulosic materials to animal feed (R. W. Detroy)
8002-20520-227	Studies on processing mustard seed meal into high-protein food products (C. H. VanEtten)
8001-20520-237	Fermentative conversion of cellulosic materials into feed and food protein (H. L. Wang)
8001-20520-238	Fermentative production of tempeh- and ontjom-type foods from soybeans for use in India (H. L. Wang)
8002-20520-239	New sorghum foods supplemented with vegetable protein (J. S. Wall)
8001-20520-241	Factors that stimulate fermentative production of carotene from cereal grains (A. Ciegler)
8001-20520-242	Indian fermented foods from U.S. soybeans (C. W. Hesseltine)

8003-20520-243	Influence of triglyceride structure on formation of geometrical isomers on soybean oil (T. L. Mounts)
8001-20520-246	Nutritional and physiological studies of soybean hemagglutinins (W. J. Wolf)
8010-20520-247	Autoxidation of unsaturated fatty acids from soybean and linseed oils (E. N. Frankel)

TECHNOLOGIES FOR FOOD AND FEED USES OF ANIMAL PRODUCTS (p. 72)

3102-20530-001	Conversion of feedlot wastes into feed supplements by fermentation with grain (G. R. Hrubant)

TECHNOLOGIES FOR INDUSTRIAL USES OF PLANT AND ANIMAL PRODUCTS (p. 75)

3102-20540-002	Production, characterization, and evaluation of cationic microbial polysaccharides (J. E. Hodge and M. E. Slodki)
3102-20540-003	Cobalt 60 initiated graft copolymers of starch (G. F. Fanta)
3102-20540-004	Lubricants from new seed oils (J. A. Rothfus)
3102-20540-005	New linseed oil emulsions and solutions for improving quality of Portland cement concrete (L. E. Gast)
3102-20540-006	Preparation of improved starch-filled rubbers (R. A. Buchanan)
3102-20540-007	Sustained release pesticide-starch products (W. M. Doane)
3102-20540-008	Immobilized enzyme treatment of papermill wastes for pollution control and reuse (K. L. Smiley and B. T. Hofreiter)
3102-20540-009	Paper machine recycling systems for pollution abatement (G. E. Hamerstrand)
3102-20540-010	Cereal-derived additives for closed white-water systems in papermaking (B. T. Hofreiter)
3102-20540-011	Isolation of a perfect stage of the yeast Candida utilis (A. I. Herman)
3102-20540-012	Condensation and vinyl polymers from linseed oil for water-based or solventless coatings (L. E. Gast)
3102-20540-013	Production and properties of enzymes for antitumor therapy (R. E. Peterson)
3102-20540-014	Refinement and chemical modification of jojoba oil (T. K. Miwa)
3102-20540-015	Linseed oil for mildew resistant aqueous paints and pesticide sprays (R. L. Eissler)
3102-20540-016	Biodegradable, nontoxic plastics additives from vegetable oil soapstocks (E. H. Pryde)

3102-20540-017	Nonpollutant paper and textile additives from cereal flours and starches (J. C. Rankin)
3102-20540-018	Starch-based copolymers for making elastomers (T. P. Abbott)
3102-20540-019	Starch-based purification aids for wastewater (W. M. Doane and R. E. Wing)
3102-20540-020	Flame-resistant polyurethane foams and biodegradable films and packaging prepared from starch (F. H. Otey and R. A. Buchanan)
3090-20541-021-C	Processing of crambe in commercial extraction equipment (K. D. Carlson)
3102-20540-022	Survey of domestic plants for rubber and hydrocarbons (R. A. Buchanan)
3102-20540-023	Chemical modification of soybean oil and its derivatives (L. E. Gast)
8003-20540-041	Polyprenoids as coenzymes for the biosynthesis of polysaccharides in enzyme systems (M. E. Slodki)
8002-20540-240	Collection of mucorales fungi in Pakistan for industrial use (C. W. Hesseltine)

TECHNOLOGIES FOR FIBER USES (p. 122)

3102-20550-001	Storage and processing of kenaf for pulp and papermaking (M. O. Bagby)

TECHNOLOGIES FOR MARKETING OF FIELD CROPS (p. 127)

3102-20590-001	Microbial spoilage in corn and grain sorghum (R. J. Bothast)

TECHNOLOGIES FOR FOOD AND FEED USES OF FIELD CROPS

A_1 Technological Objective: Reduce costs and improve markets for field crops by product innovation, increasing processing efficiency, reducing energy requirements, better nutrient retention and improved quality, safety, and pollution control.

1. Progress Report (Narrative):

15650-009

Specific Objective: Determine effects of processing on interaction with the objectionable lipid-derived flavors in soybeans to increase organoleptic qualities of soy products.

Progress: A possible solution to the flavor problem is the extraction of soy flakes with hexane:ethanol azeotrope. However, preliminary taste panel tests indicate that residual solvent levels of more than 1,000 ppm reduce flavor scores of hexane:ethanol azeotrope-extracted soy flakes. A chromatographic procedure was modified to isolate lipoxygenase-1 and lipoxygenase-2, which are possible catalysts for formation of flavor compounds, from both whole soybeans and defatted flakes. Work Unit 3102-15650-009 which terminated 8-12-76 was replaced with new Work Unit 3102-20520-023.

20520-001

a. Specific Objective: Determine the subcellular distribution and composition of endosperm proteins.

Progress: Indigenous races of maize were examined microscopically for structural and compositional features of potential interest for improvement of the nutritional or processing characteristics of domestic maize. A South American maize, Coroico, was discovered that had up to six layers of aleurone cells as opposed to a single layer in hybrid corn. Analytical data show that these cells are rich in protein with a high-lysine content. This multiple aleurone characteristic indicates the potential of improving the nutritional quality of yellow dent hybrids and has been successfully transferred to a small number of domestic lines. Studies on protein from high-amylose corn revealed a significantly higher level of lysine than that of normal corn. Since lysine

is an essential amino acid, the higher lysine protein will upgrade the feed stock produced from processing high-amylose corn starch.

b. Specific Objective: Confirm and extend quantitative data on digestibility of high-amylose starches in humans and on contribution of amylomaize to bulk content of stool.

Progress: Digestibility studies on high-amylose corn starch showed that only 89% of the starch was digested by humans, and the remaining 11% passed through as starch granules. A diet containing high-amylose starch would have the dual advantage of lower calories and added bulk. Ultrastructural studies of corn protein bodies (main site of zein) showed that after 70% alcohol extraction, bodies from high-amylose corn leave a network of undissolved protein whereas normal corn leaves only a central core. Enzymatic digestion suggests that this insoluble fraction surrounded by zein is not readily available for nutritive energy and that pretreatment of corn would increase the availability of the more nutritious protein fractions.

20520-002

a. Specific Objective: Prepare new starch derivatives with unique physical and chemical properties of interest to food processing by immobilized α-amylase.

Progress: Jet-cooked starch solutions with up to 10% starch-solids were treated with α-amylase immobilized on a phenolformaldehyde resin. The product starch, after reconstitution with water to provide 4 to 12% solids gave solutions that were relatively clear, nongelling, with viscosities only slightly less than that of untreated jet-cooked starch. The high-molecular weight fraction obtained by alcohol precipitation had properties similar to amylopectin, indicating that the immobilized enzyme acted primarily on the amylose fraction of starch. The enzyme carrier, Duolite S-761, preferentially adsorbed amylose from starch solutions allowing the immobilized enzyme to attack the adsorbed amylose. The small oligosaccharides thus formed desorb from the carrier and the process then repeats itself. This is the first known instance in which the enzyme carrier has been shown to play a paramount role in determining the action pattern of an enzyme. Alpha-

amylase attached to crab chitin did not show the selective action on amylose as described above. The α-amylose-chitin complex attacked amylopectin at about one-third the rate of the reaction on amylose. Even so, gel permeation chromatography of the products from the action of chitin-α-amylase on starch revealed the presence of fragment(s) with a molecular weight in excess of 100,000 in addition to low-molecular weight oligosaccharides up to DP-6. This product distribution is very similar to that obtained with α-amylase immobilized on Duolite S-761, and demonstrated that the enzyme bound to chitin also exhibits an exoenzymic action pattern. Strangely, enzyme on finely ground Duolite S-761 (150-200 mesh) displayed 100 times more activity and behaved in a fashion similar to chitin-α-amylase, i.e., more activity on the amylopectin fraction.

b. Specific Objective: Prepare new types of corn syrup with immobilized microbial β-amylase and a starch-debranching isoamylase.

Progress: A commercially feasible medium for making microbial β-amylase using Bacillus polymyxa cultures was developed. β-Amylase yields are about a unit/ml. The only active preparations of immobilized β-amylase were prepared by covalently binding the enzyme to porous silica with a diazonium salt. Unfortunately, this is not practical for large-scale use. Simple adsorption of the β-amylase to the carrier followed by crosslinking of the enzyme with glutaraldehyde could not be used because glutaraldehyde rapidly inactivated the enzyme.

c. Specific Objective: Improve soy milk products by degrading flatulence factors (raffinose and stachyose) with crude alpha-galactosidase in a hollow tube reactor.

Progress: Procedures for the production of the mixed enzyme extract of alpha-galactosidase and invertase from growth of Aspergillus awamori NRRL-4869 have been developed. The ratio of alpha-galactosidase to invertase is about 3:7.

The kinetics of the mixed enzyme system have been examined and "apparent" Vimax and Km values for alpha-galactosidase determined for raffinose and melibiose substrates are approximately the same. The mixed enzyme system is complex and can be partially described by the following equations:

(1) Raffinose $\xrightleftharpoons{\text{alpha-gal.}}$ Sucrose + Galactose

(2) Sucrose $\xrightleftharpoons{\text{Invert.}}$ Glucose + Fructose

(3) Raffinose $\xrightleftharpoons{\text{Invert.}}$ Melibiose + Fructose

(4) Melibiose $\xrightleftharpoons{\text{alpha-gal.}}$ Glucose + Galactose

(5) Alpha-galactose = f (glu., gal., fruc.)

(6) Invert. activity = f (glu., gal., fruc.)

Melibiose has been found in reaction mixtures and, therefore, reactions (3) and (4) are probable. Equations (5) and (6) were indicated by preliminary inhibition studies. Solution of these complicating factors will be critical for the process.

To determine exact reaction and inhibition constants it is necessary to either isolate the enzymes or change their ratio. We have begun enzyme separation and enzyme suppression studies--alpha-galactosidase is suppressed by iodine.

The results obtained from the batch studies are being applied to the continuous operation of the hollow fiber enzyme reactor. A pretreatment of the polysulfone fibers with albumin, soy whey, or soy milk was found to be necessary to prevent inactivation of both enzymes by the tubing itself.

20520-003

Specific Objective: Removal of phytic acid in soybeans to increase the utilization of trace elements in soybeans and also the solubility of soybean protein.

Progress: Based on results with 20 varieties, soybeans contained from 7.2 to 18.2 mg of phytic acid per g defatted meal, with an average of 14.3 mg, almost all of which is water soluble. When soybean slurry was incubated with Aspergillus oryzae phytase, phytic acid was completely removed after 4 hr at 37° C. In the absence of added phytase, a 50% decrease in phytic acid content was observed when whole soybeans were soaked in water for 24 hr at room temperature (23° C). Only a trace amount of phytic acid was found in the soaking water. At 55° C, about 90% of the phytic acid was removed and a significant amount of phytic acid was in the soaking water. Our results suggested a breakdown of phytic acid during soaking; however, we have not been able to demonstrate the presence of phytase in soybeans, perhaps because of its low activity. Effort was contributed toward preparation of the AID report discussed under "Research To Expand Agricultural Exports."

20520-004

Specific Objective: Continue the operation of the ARS Culture Collection.

Progress: Mycology staff members of the ARS Culture Collection continued acquisition, maintenance, and distribution of cultures and information on them; they also continued systematic studies, supportive, and original research on them. As of January 1, 1977, the Collection maintained 56,161 strains of molds, yeasts, bacteria, actinomycetes, and algae. During 1976, the Collection distributed 3,669 cultures, of which 2,057 were sent to investigators in the United States and 1,612 were sent abroad. In addition, over 2,000 separate lots (10 g each) of tempeh starters were mailed to requestors. Tempeh starters contain a high count of pure NRRL 2710 Rhizopus oligosporus spores for inoculating soybeans to make the fermented food tempeh.

The first workshop on Culture Collections sponsored by the U.S. Federation for Culture Collections was held at NRRC. ARS Culture Collection members hosted 22 visitors who attended the workshop.

Continued emphasis was placed on clarifying the systematics of yeasts, mycotoxin-producing molds, and mucoraceous fungi. Many letters (2,204) were written during 1976 in reply to requests for cultures or for information

concerning cultures and the operation of the ARS Culture Collection.

20520-005

a. Specific Objective: Collect and study isolates of Rhizopus and related molds from a wide variety of sources, especially cereal grains.

Progress: Seventy-one isolates of fungi representing Rhizopus and 13 related genera were collected and examined. These isolates came from corn, wheat, soybeans, Oriental fermented foods, soil debris, plant and animal sources. Isolates from many and varied sources will form the reservoir for further detailed studies related to improved quality of food and feed grains and to certain aspects of health.

b. Specific Objective: Examine zygosporogenesis and azygosporogenesis in selected members of Mucorales.

Progress: Gilbertella persicaria, Mucor azygosporus, and Phycomyces nitens were examined at crucial developmental stages by light microscopy, and scanning and transmission electron microscopy. Structures formed by imperfect hybridization between G. persicaria and Rhizopus stolonifer were also illustrated. The maturing azygosporangia of G. persicaria possess a continuous secondary azygospore wall that is laid down within the original gametangial wall. Within the secondary wall conical, electron-opaque warts develop. For Mucor azygosporus, the mature complex azygosporangial wall is composed of (1) remnants of the membranous outer primary wall, (2) an ornamental layer of electron-opaque, stellate, confluent warts, and (3) a fibrillar, electron-opaque tertiary layer. Cyrofractured zygosporangia of Phycomyces nitens revealed a smooth hyaline zygospore with truncate ends. A correlative study of wall layers of maturing and mature azygospores and zygospores will help clarify relationships between species and genera of the Mucorales.

c. Specific Objective: Completion of studies on Rhizomucor, an important genus from the standpoint of rennet-like enzyme production and also a potential secondary animal pathogen.

Progress: Growth of strains of Rhizomucor pusillus and R. miehei on defined media containing varying amounts of sodium chloride to present a wide range of water activity added support to the morphological data that the two entities are distinct and should be retained as distinct species. As little as 5% weight per weight addition of NaCl retarded germination of sporangiospores, extent of linear growth, and time of sporangial formation in both species when grown at the near optimum temperature of 34° C. Sporangiospore germination did not occur by the end of 7 days when as much as 15% NaCl was added to the medium whereas all strains tested gave germination within a few hours when no NaCl was added to the medium. During the compilation of morphological data and illustrations of species of Rhizomucor it was discovered that several of the strains did not fit into the current species concepts for either R. pusillus or R. miehei but comprised a new and distinct species that should be defined and compared with those species.

Comparative studies at different water activities of several strains each of Rhizopus arrhizus and R. oryzae when incubated at 25° C showed similar retardation of germination, linear growth, and sporulation with increased amounts of NaCl. This particular line of investigation did not aid in distinguishing R. oryzae from R. arrhizus nor in distinguishing strains isolated from grain sources from those isolated from animal sources.

20520-006

Specific Objective: Complete the compositional study of wild rice as related to varietal, processing, and environmental factors.

Progress: Compositional difference of grain due to strains, processing, and environment can be expected. The 34 wild rice samples studied showed variations between samples; however, these variations were not related to either strains or processing conditions. Data from elemental analyses of the wild rice samples showed some variations that may be of significance. Canadian lake rice samples have a very high level of copper (averaged 7.3 ppm vs. 3.1 ppm in the samples from Mesabi Iron Range). Wild rice samples from the Mesabi Iron Range, however, contain 25 ppm of iron as

compared to 15 ppm of iron in the samples from other regions. Therefore, the mineral composition of wild rice is influenced to some extent by the environment in which they are grown. Two commercial wild rice samples of different variety from different processors and two laboratory-processed samples, one fermented and the other not, were investigated for their protein efficiency ratio (PER), digestibility and amino acid composition. There was no significant difference in these three criteria among the four samples. The PER ranged from 1.72 to 1.82 compared to 2.50 for casein. Digestibility was also similar for the four samples, but somewhat less than that of casein. The amino acid composition of wild rice was similar to that of oats.

20520-007

a. Specific Objective: Confirm identity of neutral lipids obtained from soy protein isolate as mono- and diglycerides and complete characterization.

Progress: Conditions for silica gel column chromatography of mono-, di-, and triglyceride standards were established, and the technique can now be applied to the neutral lipid fraction separated from soy protein isolate to confirm identity and to estimate the amounts of each fraction present.

b. Specific Objective: Separate acid-sensitive fraction of soy proteins into components and characterize them.

Progress: Disc gel electrophoresis (DGE) was evaluated as a method of distinguishing between the various soy proteins. These were electrophoresed in pH 8.9 tris-glycine buffer. Gel concentrations ranged from 4 to 13%. The results were analyzed by constructing Ferguson plots (log R_f vs. gel concentration) from which retardation coefficients and free mobilities were obtained. Soy proteins were shown to be much more complex than apparent after DGE at only a single gel concentration. Although no more than 20 proteins were discerned on any one gel, 28 were distinguished among all the gels. Separation and characterization of the acid-sensitive fraction can now proceed using DGE to monitor progress.

c. Specific Objective: Determine distribution of cadmium in anatomical parts of the soybean and its fate during fractionation in cooperative work with Food and Drug Administration (FDA) on soybeans grown on sewage sludge-treated soil.

Progress: A control and a sample of soybeans high in cadmium content (grown on sewage sludge-treated soil) were fractionated into hulls, cotyledons, and hypocotyls which were analyzed for cadmium and lead by FDA. Heavy metals were not distributed uniformly. The hulls from beans with a high cadmium content made up 7% of the seed but contained about one-fifth of the total cadmium. Both soybean samples have been processed into defatted flakes and oil and the control sample of defatted flakes was further processed into protein concentrates and isolates. These samples and associated byproducts are being analyzed for lead and cadmium to learn whether the heavy metals remain with the protein fraction or are removed during processing.

d. Specific Objective: Evaluate the effects of ultrasonic treatment on the physical properties of soybean proteins.

Progress: Soybean proteins prepared by ultrasonic or stirring extraction were compared by gel filtration and disc gel electrophoresis. Gel filtration separated proteins from defatted flakes prepared by stirring extraction into 8 peaks of varying molecular weights. In contrast, the proteins from either autoclaved or unautoclaved flakes prepared by ultrasonic extraction yielded only one major peak with a low K_d distribution coefficient, which indicated protein aggregation. Results from gel filtration also suggested that the proteins contained low molecular weight contaminants. Comparable studies by disc electrophoresis, likewise, indicated that soybean proteins given ultrasonic or autoclaving treatments aggregated into a larger molecular size than proteins not so treated.

e. Specific Objective: Complete evaluation of carbohydrate analysis as a possible method for determining soy protein in meat products and explore terminal amino acid method in more detail to determine feasibility as a tag for soy protein.

Progress: Total carbohydrate content of soy flours, protein concentrates, and isolates have been determined by GLC analysis of the alditol acetate derivatives of the sugars after acid hydrolysis of the samples. In addition, oligosaccharides were measured by high-pressure liquid chromatography. Carbohydrate analysis of meat-soy mixtures indicates that meat-soy flour and meat-soy concentrates can be estimated with an accuracy of about +10% but the technique fails with meat-isolate mixtures because of the low level of carbohydrates present. Analyses of N-terminal amino acids of soy and meat proteins by the phenyl isothiocyanate method (Edman procedure) indicate N-terminal glycine, valine, and leucine-isoleucine occur in soy flour and soy isolates at levels 3 to 8 times higher than in beef steak. Mixtures of soy flour and beef were analyzed and results are being evaluated statistically to determine accuracy of the method and potential as a method for estimating the amount of soy in such mixtures.

20520-008

a. Specific Objective: Establish growth conditions for thermophilic Actinomycetales on solid substrates and screen available thermophiles for release of bound cellulose.

Progress: Agricultural residues, prepared by grinding and milling were used as substrates for cellulolytic microorganisms for development of possible animal feedstock. These substrates, cornstalks, husks, wheat straw, and alfalfa, were digested at 55° C with crude Trichoderma viride cellulase preparations. The "ligno-cellulose" residues from this treatment (no free cellulose) were used to evaluate 36 thermophilic (SSC) actinomycetes for their ability to degrade lignocellulose. These thermophilic organisms, isolated from active compost piles, utilize the ligno-cellulosic residues sparingly with little or no cellulase activity (filter paper assay) or release of bound cellulose. Cellulase-digested alfalfa residue was inhibitory to all of the thermophilics when incorporated into a combined N medium. The inihibitor was extractable by either hot water or alcohol.

b. Specific Objective: Determine value of organisms selected above on whole agricultural residues.

Progress: The thermophilic actinomycetes are being tested on solid substrates of agricultural residues on a continuous roller-drum for ability to grow and/or produce cellulase and "lignocellulase" activities.

20520-009

a. Specific Objective: Develop new methods of parching wild rice.

Progress: Although preliminary studies on using a corn roaster for parching were promising, more extensive work showed that the particular roaster we used was not suitable. This machine, designed for corn and soybeans, would require a complete redesign to be applicable for use with wild rice. Several other approaches to wild rice parching are, therefore, under study.

b. Specific Objective: Study alternate treatments for green wild rice prior to parching to better prepare rice for subsequent processing.

Progress: It was established that steaming the rice to gelatinize the starch is a necessary part of the wild rice process. Moisture content of green rice should be 40-50% in order to satisfactorily condition the starch. This can be readily accomplished in a continuous jacketed mixer at a temperature of about 155° C. In addition to eliminating undesirable white centers in the finished rice, steaming appears to aid subsequent dehulling and other milling operations.

c. Specific Objective: Determine where wild rice breakage occurs.

Progress: A survey was conducted to determine where breakage of wild rice occurs. Samples were collected at various stages of harvesting and processing of the rice, and broken and unbroken kernel counts were determined. It was shown that breakage was negligible until the rice entered the plant. Most of the breakage occurring was in the dehulling and scarification steps.

20520-010

Specific Objective: Study microscopic structure and chemical composition of wild rice pericarp and those tissues which are pigmented.

Progress: Pigmentation in the pericarp ranged from green to dark brown and is localized primarily in a single layer of subepidermal cells. Both brown and green pigments can be found in the same cell as spherical bodies. At maturity, the brown pigments predominate. These pigments are derived from chlorophyll, protein, and cell remnants. The brown pigments are believed to be phaeophytins formed from chlorophyll. Smaller amounts of similar pigments are localized in other epidermal cells and in the cross cells. The seed coat which is the protective layer of the kernel is composed of two semi-permeable suberized membranes. The two membranes are separated by cell remnants and deposits of polyphenolic compounds. The inner seed coat membrane is composed of two distinct layers. Other structural adaptations include heavily cutinized pericarp cell walls and a thick layer of wax on the outer epidermal wall of the pericarp.

20520-011

a. Specific Objective: Develop a specific assay for the C_1 or swelling factor of the Trichoderma viride cellulase complex.

Progress: The specific value of the increase measured by the Solute Exclusion Technique depends not only on the ratio of the C_1 factor to cellulose, but also on the specific nature of the cellulose. Initial studies indicate that the ratio of cellulose solubilization in unsaturated digests of T. viride cellulase with Avicel or filter paper sensitive to C_1 swelling) to that of cotton (insensitive to C_1 swelling) is a relative measure of C_1 induced swelling.

b. Specific Objective: Scale up and refine the Sephadex adsorption isolation of the cellobiase cofactor.

Progress: Two 20-liter T. viride cellulase preparations were prepared by fermentation of Solka-floc with T. viride fungi under conditions expected to give high yields of the T. viride cellulase enzyme and its cofactors. Portions of each preparation were stored at 40° C, frozen at -18° C, lyophilized at -18° C, and as a 60% acetone precipitate and solubles fraction at -18° C.

c. Specific Objective: To isolate and study the chemical and physical properties of hemicellulose of pith from the stalks of corn, sorghum, and sugar cane.

Progress: Methods were developed to separate pith from fiber, purify pith, and to isolate hemicellulose from the purified pith. Pith has been obtained from stalks of corn, sorghum, sugar cane, and sunflowers.

d. Specific Objective: To devise methods suitable for the analysis of hemicellulosic and fiber material derived from agricultural byproducts such as the pith from the stalks of corn, sugar cane, or sorghum, and to determine the compositional differences in the hemicellulose and fiber from the various agricultural residues.

Progress: Agricultural residues and hemicelluloses isolated from the pith are being analyzed by gas chromatographic separation of the alditol acetate derivatives. The compositional differences in the hemicellulose and fiber fractions are being compared. Several residues have been examined with xylose contents in the range of 20%.

20520-012

a. Specific Objective: Develop and evaluate new cereal food products.

Progress: The effect of fortifying degermed cornmeal with defatted peanut flour (DPF) was evaluated by studying flavor, stability, product uses, and nutrition. Flavor was acceptable in gruels made from blends fortified with up to 50% DPF. Changes in flavor, peroxides, free fatty acids, and available and added lysine after storage for 2, 6, and 12 months at 49°, 37°, and 25° C, respectively, were evaluated and found satisfactory. Leavened and unleavened breads were acceptable in blends containing up to 20% DPF. Protein efficiency ratio (PER) increased from 0.3 to 1.4 with 15% DPF fortification. A PER of 2.0 resulted with the addition of 0.18% L-lysine HCl to the blend. Specifications have been drafted for peanut-fortified corn meal and for peanut-soy-fortified corn meal. In cooperation with the University of Hawaii, an investigation into alternate uses for taro root showed that wet milling

of the roots materially reduced objectionable crystals of calcium oxalate and produced a satisfactory starch product. High lysine grain sorghum can be dry milled to produce grits, flour, germ, and feed. Floury character of the endosperm results in reduced yields of grits. Lysine appears to concentrate in the germ and feed fractions.

b. Specific Objective: Determine dry milling characteristics of several different corns.

Progress: Yellow dent corn was shelled at four harvest moisture levels, two sheller damage settings, and then dried at five temperatures ranging from 30° to 300° F. Yields of prime products (i.e., grits, low-fat meal, and flour) from degerminated roller-milled stock were highest using corn dried naturally or at the lowest heated air temperature of 120° F. Fat contents of the prime products were lowest at the intermediate air drying temperature of 180° F and increased as drying temperature was either raised or lowered. While unexpected, this corroborates previous findings. Corn harvested at higher sheller damage settings showed reduced prime product yields when roller milled compared to corn picked and shelled at low damage settings; fat content of prime products was unaffected by sheller damage.

Roller milled germ fraction yields were highest from corn dried at 300° F and yields were decreased in corn dried at lower temperatures. Germ fat content varied inversely with corn drying temperature, fat being highest in roller milled fractions from harvested corn dried with the lowest air temperatures.

Roller milling yields and fat contents did not correlate well with whole kernel stress cracks, kernel breakage, or kernel weight.

20520-013

a. Specific Objective: To improve food and feed acceptability by providing antioxidants for corn and other vegetable oils.

Progress: Partition of a stable, dark oil extracted from ammoniated corn gave a basic fraction (0.7%) which contained 8 compounds, 4 of which gave a positive test for sugar-derived imidazoles. The acidic fraction (0.75%) contained at least 6 components separable by

TLC. Products from the reaction of valine alone and valine + glucose (or dihydroxyacetone) in heated corn oil were compared with those extracted from the dark corn oil. When ethylene glycol was substituted for corn oil as the heating medium, the products were different. Evidence for nonenzymic browning products in the stable oil from ammoniated corn was obtained. A phenolic-type antioxidant, $C_{22}H_{20}O_6$, isolated from a sugar-amine reaction was characterized as a dimer of 4,5-dihydroxy-3,6-dimethylindenone. The monomer has been synthesized and only the dimerization step remains to prove the structure of the aromatic, sugar-derived molecule. An enolic furanone, proposed by others as a nonenzymatic browning product but never isolated and characterized, was produced by hydrolysis of a key browning reaction intermediate. As determined by mass and NMR spectroscopy its structure is 5-hydroxymethyl-4-hydroxy-2-methyl-3(2H)-furanone. Work on synthesis of this compound remains to be completed.

b. Specific Objective: To provide new alditols and alditol esters of fatty acids for use as noncariogenic and low-caloric sweeteners and as surface-active agents in foods.

Progress: Borohydride reduction of the keto-disaccharides, maltulose and lactulose, produced sirupy alditols that were sweet. Analysis by GLC did not resolve the mixture of alditols. Synthesis of 4-O-α-D-glucosylmannitol is in progress to determine its sweetness relative to 4-O-α-D-glucosylglucitol (maltitol), a commercial sweetener.

c. Specific Objective: To improve food acceptability by providing suitable carbohydrate flavor-complexing agents.

Progress: The molecular complexes of maltulose with methyl cyclopentenolone and maltol, which were somewhat sweeter than maltulose, were found not to be stable in aqueous media. They could be prepared under anhydrous conditions but not when water was present. Derivatives of fructose were synthesized in both 5- and 6-membered ring forms to determine the ring form of the fructose moiety of crystalline maltulose. Significant differences in the C-13 NMR spectra of the different ring forms of fructose were defined. Preparation of the corresponding maltulose and lactulose derivatives gave mixtures of

anomeric and isomeric ring forms due to a rapid equilibration in the preparative media. The C-13 NMR spectra of the keto-disaccharides, even in dimethyl sulfoxide wherein the mutarotation rates are minimal, showed that many different isomeric forms had developed in the time required for dissolution and determining the spectra.

d. Specific Objective: To determine the structural factors in hydroxylic compounds that are involved in the sweet taste response.

Progress: To extend a previous finding that introduction of an oxygen atom into the ring of nonsweet cis-1,2-cyclopentanediol introduced a slight sweet taste into 1,4-anhydroerythritol, the same procedure was applied to nonsweet 1,2-cyclohexanediol. Synthesis of cis,trans-3,4-dihydroxytetrahydropyran provided a slightly sweet (still bitter) mixture. Thus, both 5- and 6-membered cyclic glycols containing a heterocyclic oxygen atom were shown to be sweet. The cis and trans isomers of the pyran glycol are now being synthesized. To model natural, intensely sweet bis-glycosides with a central hydrophobic aglycone, mono- and bis-glycosides of 1,4-butanediol, 1,6-hexanediol, 2,5-hexanediol, and isosorbide (1,4:2,6-dianhydro-D-glucitol) were synthesized. The bis-β-glucosides of the alkanediols were tasteless. A simple procedure was found for preparing both isosorbide and isomannide using acidic ion exchange resin as catalyst. Six of the eight steps required to synthesize glycosidic analogs of a sweet compound (phyllodulcin) found in certain species of hydrangea have been completed.

e. Specific Objective: To develop a rapid analytical method for determining hemicelluloses and polyuronides in food fiber materials such as bran, hulls, cereals, protopectin, and mucilages.

Progress: A rapid gas-liquid chromatographic method for estimating the amount of each sugar in hemicellulose hydrolyzates was developed. To determine uronic acids in the hydrolyzates, a simple new colorimetric method was developed which is also applicable to glucono-1,5-lactone. The new GLC method reduced analysis time of the currently used method (18 hr) to 2.25 hr. Sugars that were completely separated in a single mixture as aldononitrile acetates were xylose, arabinose, ribose, rhamnose, galactose, mannose, glucose, and 2-deoxyglucose.

f. Specific Objective: To determine the complexing behavior of certain food-fiber polysaccharides with nutrient minerals to provide a better understanding of the role of plant fiber in maintaining human health.

Progress: The subject of polysaccharide-metal complexes was reviewed (see publications). Work toward this objective was delayed to allow further investigation of principles underlying aldose to ketose isomerizations in the presence of column-bound aluminate. Kinetic studies indicated that hydroxide ion is the isomerization catalyst and that almost negligible amounts of mannose are formed. Starting with glucose, yields of fructose were raised to 70% (48 hr, 25° C) on an aluminate column of lowered hydroxyl content and increased aluminate substitution (81%). The maximum yield of fructose from glucose on hydroxide resin in the absence of aluminate is about 28%. Relative complexing abilities of 19 carbohydrates with sodium aluminate were determined in solution. None was more strongly complexed than fructose; glucose was only one-fourth as reactive. Configuration and conformation of sugars and alditols were shown to be important factors in the complexing reaction. Maltose was isomerized to maltulose on the aluminate column more rapidly than glucose to fructose; a 63% yield was reached in 8 hr at 25° C. Further reaction produced glucose and fructose and less maltulose.

20520-014

a. Specific Objective: Characterize autoxidation products of linolenate-containing materials as a means of determining autoxidation mechanism.

Progress: A gas chromatography-mass spectrometry (GC-MS) computer summation approach was developed to analyze quantitatively the isomeric hydroperoxides in oxidized oleate and linoleate, as a basis for study of oxidized linolenate and soybean oil. This method was standardized by using 8-, 9-, 10-, and 11-hydroxystearate synthesized in collaboration with workers at Queen Mary College, University of London, where the project leader was invited to spend a year on a visiting research fellowship. By this method, it is now possible for the first time to analyze the origin of hydroperoxides formed in autoxidized mixtures of oleate and linoleate.

This method is expected to be applicable to the determination of the origin of hydroperoxides in mixtures of unsaturated fatty esters similar to those occurring in soybean oil and other vegetable oils.

Structural studies by high-pressure liquid chromatography (HPLC) were carried out on hydroxy derivatives from oxidized oleate and linoleate to complement identifications achieved by GC-MS. New knowledge on the proportion of primary products and on the secondary products of oxidation will help determine the contribution of these precursors to off-flavors and odors in soybean oil.

b. Specific Objective: Prepare and evaluate polymer-supported chromium carbonyl catalysts as cis-bond forming hydrogenation catalysts.

Progress: Conventional and macroreticular styrene-divinyl benzene copolymers, poly(vinylbenzoate), and poly(ethylene terephthalate) resins were complexes with chromium carbonyl. The complexed poly(vinyl benzoate) was shown to be more active than the polystyrene catalysts for the cis hydrogenation of methyl sorbate, conjugated linoleate, and soybean esters. Both types of catalysts could be recycled once or twice but the loss of activity and of chromium permitted only about three recycles.

20520-015

a. Specific Objective: Develop protein-enriched foods based on wheat gluten.

Progress: A series of polysaccharides produced by different microorganisms were tested for their effect on gluten protein properties. Polysaccharides from cultures B-1973 and Y-1401 as well as xanthan gum react with gluten and other proteins in very dilute solution so as to insolubilize the proteins and modify their physical properties. These observations may provide means of recovering soluble proteins in wheat gluten production processes and altering the texture of gluten in protein supplemented products.

Because of the effect of the polysaccharides on the isolated protein, various polysaccharides were added to soft wheat flour doughs to examine their action on dough properties. Polysaccharide B-1973 and xanthan gum increased mixing time requirement and dough stability

in the farinograph. Three commercial carrageenans (gums of seaweeds) increased dough stability at levels as low as 0.1 percent of flour weight.

b. Specific Objective: Determine relationship of wheat proteins to functionality.

Progress: N-terminal amino acid sequence analyses of several isolated wheat gliadin proteins indicate that all α, β, and γ gliadins are probably derived by modification of a single gene in wheat's most primitive ancestor. Comparisons of established N-terminal sequences suggests that γ gliadins from different wheat varieties may vary while α gliadins do not; this finding suggests that differences in wheat flour performance between varieties may be related to differences in γ gliadins. Glutenin subunits seem to be less amenable to sequence determination than are gliadins; preliminary results indicate significant differences in amino acid sequences of glutenins from those of gliadin proteins.

Molecular weights of isolated wheat glutenin proteins were determined by ultracentrifugal analysis in dissociating solvents such as 8 M urea or 6M guanidine hydrochloride. Values obtained for various fractions are lower than those found by use of SDS electrophoresis. Evidently differences in conformation of glutenin proteins compared to globulins used for standards account for the low molecular weight obtained by SDS electrophoresis.

c. Specific Objective: Produce high protein fractions from oats and wheat suitable for protein supplements in foods.

Progress: Flours of two varieties of high-protein soft wheat were successfully air-classified into high yields of high-protein fractions. One high protein wheat flour with 16 percent protein yielded a combined high protein fraction containing 29 percent protein and constituting 33 percent of the flour.

Oat flour was separated into protein- and starch-rich fractions by density differences relative to a Freon-hexane solution of suitable density. Floating fractions with over 70 percent protein content were obtained which represented 40 percent of the protein in the oat flour. The protein-rich fraction had good hydration capacity.

20520-017

a. Specific Objective: Investigate action of copper catalysts in laboratory scale continuous hydrogenation of soybean oil to obtain kinetic information useful in larger scale continuous hydrogenation.

Progress: The study of continuous hydrogenations with nickel catalyst in our laboratory-scale Teflon tubing reactor has been completed. The reaction has been satisfactorily modeled using first order kinetics, and a statistical study of effect of temperature, oil flow rate, and hydrogen flow rate has been completed. Reaction rates increase with temperature. trans Content and degree of hydrogenation increase with temperature and decrease with increased oil flow. Hydrogen flow rate has little effect over the range studied. With copper catalyst it was necessary to use a longer reactor to secure greater residence time and to modify the apparatus to give good flow of the oil catalyst slurry.

b. Specific Objective: Investigate batch high-pressure hydrogenation of soybean oil to determine products formed and applicability of high pressure continuous hydrogenation of soybean oil.

Progress: Soybean oil has been hydrogenated with copper catalyst at pressures of 500, 1,000, and 3,000 psi; at temperatures of 110, 130, 150, and 170° C; and at catalyst concentrations of 0.05, 0.1, 0.2, and 0.4% copper. Overall reaction rate is increased at higher pressures and lower concentrations of copper are needed for the higher reaction rate. Conjugated diene is eliminated as a measurable product in the hydrogenated oil. Contrary to previous observations, based on nickel catalysts, which predicted that selectivity decreased with increased pressure, the high selectivity of copper catalysis is retained at high pressures.

c. Specific Objective: Operate and collect data using our new fixed-bed catalytic reactor to establish a reproducible process to convert soybean oil to marketable product in good yield while retaining nutrient quality.

Progress: Continuous fixed catalyst bed hydrogenation was studied in two areas, namely (1) operation of a large continuous convertor at conditions found favorable with a smaller continuous convertor; and (2) work on a reliable technique to pre-reduce or activate the

catalyst. The large continuous convertor was operated at 120° C and 50 psig for the partial hydrogenation of soybean oil. The hydrogenated oil product contained 4% triene and 45% diene with no increase in saturates. The inability to reduce the triene to zero was attributed to improper pre-reduction of the catalyst and/or inefficient flow distribution through the catalyst bed. An attempt was made to determine the degree to which a catalyst is pre-reduced by measuring the water formed during the catalyst reduction, by the x-ray diffraction analysis of the reduced catalyst, and by the composition of an oil hydrogenated with the reduced catalyst. For this study, a 2-1 autoclave was used in which was suspended a perforated basket containing the pelletted catalyst. This apparatus allowed removal of the oil product and water without removal or deterioration of the catalyst. The x-ray results were inconclusive.

20520-018

a. Specific Objective: Develop protein concentrates from corn and sorghum.

Progress: An alkaline extraction process was used to produce protein concentrates from ground normal and high-lysine sorghum grains. Optimum protein recovery was obtained with two extractions at pH 11.9 (solvent to meal, 6:1). Starch and bran were removed from the dispersions, and protein recovered by precipitation at pH 5.0. Protein content of concentrates varied between 48 and 60% and lysine content varied from 3.1 g per 16 g N for normal sorghum concentrate and 5.4 g for high-lysine concentrate. Although the concentrates were water-insoluble at pH 3.5 to 5.8, they were 90% soluble from pH 8.7 to 10.8. The high-lysine sorghum protein isolate had good functional properties including water absorption and fat emulsification.

b. Specific Objective: Develop protein-rich flour from wet-milled corn germ.

Progress: Flavor and shelf life of a defatted and purified corn germ flour prepared from wet-milled grain were enhanced by extracting the residual bound lipid with hexane-ethanol azeotrope. The resulting high-protein flour (23%) also contains 10% crude fiber which suggests its use as a source of dietary fiber. A large amount of the defatted and purified wet-milled corn germ was prepared for nutritional evaluation.

c. Specific Objective: Establish structure of corn and sorghum proteins.

Progress: Reduced corn glutelin protein contains an alcohol-soluble (ASG) fraction that is high in methionine and low in lysine. Upon dialysis against water, the alcohol soluble-reduced glutelin separated into water-soluble and water-insoluble fractions. The water-insoluble fraction contains five times as much methionine as the soluble protein. The water-soluble fraction resembles zein in M.W. as determined by SDS electrophoresis but has different mobilities in polyacrylamide gel electrophoresis; the water-insoluble fraction has the opposite properties. Thus, neither fraction is identical to zein in all characteristics. The *su1* gene enhances the amount of water-insoluble (ASG) protein fraction as well as methionine in inbred grains containing this mutant gene.

20520-019

a. Specific Objective: Perform genetic hybridization between species of *Debaryomyces* and *Pichia* to clarify species limits.

Progress: Auxotrophic mutants were induced in 20 strains from 12 allied species in the yeast genera *Debaryomyces* and *Pichia* which are common in soil and causes of food and feed spoilage. Mutants were characterized for their biochemical deficiencies and pairings between mutants are presently being studied.

b. Specific Objective: Determine relatedness of species of *Pichia* and *Candida* through studies of nuclear DNA.

Progress: DNA was isolated and purified from species in the *Pichia rhodanensis* group. Comparisons of % guanine + cytosine (G+C) revealed one new species in the group under study. This new species, to be scientifically described in a future publication, differed from *P. rhodanensis* by 7% G+C. Furthermore, the species was not genetically interfertile with *P. rhodanensis* or other closely related species.

20520-020

Specific Objective: To continue operation of the ARS Culture Collection.

Progress: Three-hundred yeast isolates from swine waste-corn fermentation were identified. Yeasts very similar to Candida krusei and C. valida were the most prevalent types. Preliminary studies indicate that Corynebacterium and Arthrobacter-like bacteria were the predominant bacteria found during the initial phases of the swine waste-corn fermentation.

Approximately 350 bacterial isolates from the swine waste-corn fermentation were identified; the predominant species was Lactobacillus fermentum.

20520-021

a. Specific Objective: Relocate minirefinery, incorporate new sensors, and process crude soybean oils for organoleptic evaluation.

Progress: The minirefinery was totally relocated and reconstructed. The computer input system was revised from a digital to an analog signal which permits a wider dynamic range for the minirefinery data. New programming was implemented for plots of raw data and final refinery data and for new transducers. A new sensor for the measurement of iron or conductive solutions was developed based on radio frequency using a grid-dip meter. Degummed oil processed through the three stages of the minirefinery (alkali refining, bleaching, and deodorization) yielded a processed oil having flavor inferior to batch processed oil. Oil collected after the bleaching stage was deodorized in all glass batch deodorization equipment; the flavor of the oil produced was acceptable. Refined and bleached oil was processed through the continuous deodorization stage of the minirefinery, but the flavor of the oil was unacceptable. A new deodorization stage was designed and installed in the minirefinery. Testing of this new stage is incomplete.

b. Specific Objective: Organoleptic evaluation of edible oils prepared using new refining techniques.

Progress: Crude soybean oil was degummed using standard water degumming procedures or procedures involving a predegumming step using phosphoric acid. Each of the degummed oils was refined by (a) alkali, (b) steam. Flavor evaluations of the processed oils indicated that only the water degummed, steam refined oil had a poor initial flavor. Phosphoric acid treated oils showed

superior flavor stability relative to the water degummed oils. There was no significant difference between the steam refined and caustic refined, phosphoric acid treated oils. The steam refined oils had free fatty acid contents of 0.2 ppm which is considerably higher than that for alkali refined oils.

c. Specific Objective: Evaluate effect of damage to soybeans occurring during commercial handling, transportation, and storage.

Progress: Analysis of soybean sampled from an export shipment indicated that the oil from split beans had a higher iron content and free fatty acid content than the oil from whole beans of the same lot. A shipment of "identity preserved" soybeans have been sampled from barges and a unit train prior to shipment from New Orleans and after arrival in Tifton, England. Samples were received in sufficient quantity to permit extraction of adequate amounts of oil for subsequent processing and organoleptic evaluations.

d. Specific Objective: Evaluate the effects of hydrogenation and added antioxidants on the flavor and oxidative stability of soybean oil.

Progress: The flavor stability of unhydrogenated soybean oil was not improved by added antioxidants or by hydrogenation. When antioxidants were added to hydrogenated oils, the flavor stability of stored samples was improved relative to unhydrogenated oils with antioxidants. Oxidative stability was improved by both antioxidants and hydrogenation.

20520-023

a. Specific Objective: Determine effect of lipoxygenase activity on the oxidation of soybean phosphatidylcholine (SPC).

Progress: Lipoxygenase isoenzymes capable of oxidizing free and esterified unsaturated fatty acids, were isolated from soybeans. These isoenzymes, however, were unable to oxidize SPC, except in the presence of a lipase. Research has established that lipase liberates an unsaturated fatty acid moiety from SPC which can now be oxidized by the lipoxygenase enzymes. Apparently, SPC oxidation in defatted fatty flakes can occur only in the presence of a performed lipohydroperoxide.

b. Specific Objective: Determine content of nondigestible residue (dietary fiber) of soybean products, wheat and corn bran, and isolate.

Progress: Soybean and cereal products were digested with pepsin and pancreatin, to obtain an insoluble nondigestible residue (NDR) and a soluble filtrate. The NDR content as percent of dry matter was as follows: corn bran, 93.7; soybean hulls, 74.9; wheat bran, 45.7; and defatted soy flakes, 7.6. Preliminary values for acid-detergent fiber (obtained by chemical methods that measure primarily cellulose and lignin) were much lower than those obtained after hydrolysis with intestinal enzymes. Over 95% of the protein in defatted flakes was digested by the enzymes compared to only 80% in soybean hulls. This work is being done in conjunction with project 20900-2 "Composition and properties of cereal grain fibers," under "Food and Nutrition Research."

c. Specific Objective: Determine storage stability of soy products prepared by hexane:ethanol azeotrope extraction.

Progress: Previous research had established that with the proper control of azeotrope extraction and toasting, flavor scores of defatted soy flour and concentrates prepared from the azeotrope extracted flakes approach the blandness of wheat flour. Storage stability was good. There were no significant differences in flavor scores of azeotrope-extracted flakes stored at 6 months at 100° F and zero time samples stored at 0° F for the same length of time. The soy protein concentrates also exhibited very good storage stability qualities since odor and flavor scores did not change significantly even after storage for 1 year at 100° F.

d. Specific Objective: Evaluate nutritional value of soy protein products in long-term feeding trials with rats.

Progress: Growth of weanling rats fed defatted soy flour, concentrate, and isolate was equal to that of rats on a casein-based diet up to about 100 days of feeding. After this period, growth rate with the casein diet was much greater than that with soy flour. Rats fed soy protein isolate ceased to grow, whereas, those fed soy protein concentrate lost weight. In a repeat trial, vitamins and minerals were added to casein and soy concentrate diets after 149 days on test. Rats receiving the concentrate diet supplemented with vitamin B_{12} responded immediately during the first

week. The rate of growth was much greater than with the unsupplemented soy or even the casein control. Vitamin B_{12} has no effect on growth of rats fed casein. Other vitamins and minerals had little or no effect on growth. These data indicate that after prolonged feeding vitamin B_{12} stores are depleted and growth rate decreases. Further work is required to determine if this effect is a vitamin deficiency or an increased need.

2. Progress Reports (Achievements):

15650-009

Publications:

HONIG, D. H., K. WARNER, AND J. J. RACKIS. Toasting and Hexane:Ethanol Azeotrope Extraction of Defatted Soy Flakes: Flavor Evaluation of Resulting Flours, Concentrates, and Isolates. J. Food Sci. 42 (1976):642-646.

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RACKIS, J. J. International Research on Soybeans. Presented at International Institute for Tropical Agriculture, Ibadan, Nigeria, May 1976.

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3. Plan of Work:

20520-002

a. Specific Objective: Complete determination of reaction and inhibition constants for the various reactions of alpha-galactosidase and invertase with raffinose and intermediate products.

Plan of Work: By enzyme separation, enzyme suppression, or altering enzyme production methods, change the ratio of alpha-galactosidase to invertase and thus provide the means to determine the needed reaction constants.

b. Specific Objective: Model the kinetics of the soluble batch enzyme reaction system.

Plan of Work: Using data from (a) above, derive equations descriptive of the system and test the model for suitability of fit.

c. Specific Objective: Model a continuous feed hollow fiber reactor for the enzyme system.

Plan of Work: Mathematical models are given in the literature for continuous feed hollow fiber reactor using simple first order enzyme reactions. These must be adapted to our more complex reaction system using the equations from (b) above. Equation for enzyme decay may be needed and the model will be tested and refined as continuous reactions are run.

This project terminates in May 1977. It will be replaced by a new project with more basic objectives.

20520-003

Specific Objective: Continue to investigate the removal of phytic acid in soybeans to improve the nutritional quality of the seeds.

Plan of Work: The optimum conditions for removal of phytic acid in soybeans will be investigated. After phytic acid removal, the solubility of soybean protein will be tested. The presence of phytase in soybeans will be verified.

20520-004

Specific Objective: To continue the operation of the ARS Culture Collection.

Plan of Work: The staff of the ARS Culture Collection will continue its long-term operations in collecting, classifying, preliminary evaluating, maintaining, and distributing microbial cultures for research and development involving cereal microbiology as it pertains to food, feed, and health.

20520-005

a. Specific Objective: Continue collection and study on isolates of Rhizopus and related molds from a wide variety of sources, especially cereal grains.

Plan of Work: Further isolates will be examined to determine their cultural, morphological, and biochemical characteristics.

b. Specific Objective: Examine zygosporogenesis and azygosporogenesis in other selected members of the Mucorales.

Plan of Work: Correlative light, transmission electron-, and scanning electron-microscopy studies will be carried out. Freeze-fracture techniques will be used in certain instances. Results of the studies will clarify further relationships between species and genera of the subject microorganisms.

c. Specific Objective: To determine the position and distinctness of the newly discovered species.

Plan of Work: Compare isolates of Rhizomucor and related Rhizopus-like species with those of the new entity in respect to their cultural, morphological, and certain biochemical characteristics.

20520-007

a. Specific Objective: Isolate additional quantities of neutral lipids from soy protein isolate and quantitatively determine the amounts of mono-, di-, and triglycerides present.

Plan of Work: Alcohol-extractable materials from soy protein isolate will be fractionated on activated magnesium silicate columns to obtain the neutral lipid fraction which will then be separated into mono-, di-, and triglyceride fractions by silica gel column chromatography.

b. Specific Objective: Separate flavor compounds from soy protein acid-sensitive fraction (ASF), and elucidate the nature of their binding to protein.

Plan of Work: After exhaustively dialyzing ASF to remove extraneous material, detergents will be used to extract tightly held compounds. Finally mild hydrolysis with HCl in dimethylformamide will be employed to remove covalently linked compounds. Both the detergent dissociated material and the hydrolysis products will be examined for carbonyl compounds arising through oxidative linolenate degradation.

c. Specific Objective: Determine the distribution of cadmium as a result of fractionating soybeans of high cadmium content into oil, meal, protein concentrates, and protein isolates.

Plan of Work: Soybeans with high cadmium content (grown on sewage sludge-amended soil) obtained from the Food and Drug Administration will be processed into protein concentrates and protein isolates. The protein products and the resulting byproducts (whey and insoluble residue) will be sent to FDA for trace metal analysis. Modifications of processing techniques to ensure against trace metal contamination from outside sources and against metal losses will be evaluated.

d. Specific Objective: Fractionate proteins obtained by ultrasonic extraction of autoclaved and alcohol-treated soybean flakes to gain insight into the cause of increased extractability.

Plan of Work: Make protein extracts by ultrasonic treatment of autoclaved and alcohol treated soybean flakes and separate the proteins by gel filtration and disc gel electrophoresis. Separations will be compared with comparable fractions obtained by stirring extraction of treated and untreated flakes.

e. Specific Objective: Explore practical procedures of amino-terminal analysis as a feasible approach to the determination of the amounts of soy in soy:meat mixtures.

Plan of Work: Methods other than the phenyl isothiocyanate technique will be evaluated as potentially simpler ways for measuring the amino-terminal residues in soy:meat mixtures.

20520-008

a. Specific Objective: Evaluate naturally occurring thermophilic microorganisms on solid substrate-agricultural residues, wheat straw, and feedlot waste fiber for growth, cellulase activity, and degradation of lignocellulose.

Plan of Work: Isolate thermophilic microorganisms from active compost samples and evaluate their abilities to utilize lignocellulosic residues. Determine growth conditions; temperature, relative humidity, agitation, and type of substrate. Determine lignin and cellulose contents of agricultural residues before and after microbial growth. Determine cellulase activity using filter paper assays.

b. Specific Objective: Biodegradation of lignocellulosic agricultural residues by mesophilic Basidiomycete fungi (mushrooms).

Plan of Work: Evaluate growth of Basidiomycete fungi upon wheat straw and/or feedlot waste fiber, with and without nitrogen amendments. Chemically assay for lignin and cellulose degradation. Measure growth with regard to various parameters: relative humidity, aeration, agitation, substrate size, and temperature. Estimate fungal biomass yields on fermented lignocellulose residues.

20520-009

a. Specific Objective: Develop new methods of parching wild rice.

Plan of Work: Study alternative procedures for parching, such as microwave, micronizing, and the use of various types of dryers and other equipment for parching.

b. Specific Objective: Determine methods for reducing breakage in wild rice.

Plan of Work: Investigate the occurrence of kernel breakage in various steps in the processing of wild rice, i.e., parching, dehulling, scarifying, and grading, and develop procedures for reducing such breakage.

c. Specific Objective: Establish uniform quality parameters for wild rice.

Plan of Work: Study the effects of various processing of conditions on quality parameters of wild rice, such as flavor, color, cooking properties, etc.

20520-011

a. Specific Objective: Develop a specific assay for the C_1 factor (swelling) of the Trichoderma viride cellulase complex and scale up the Sephadex adsorption isolation of the cellobiase factor.

Plan of Work: Studies involving the correlation of the C_1-induced swelling of Avicel and cotton measured by the Solute Exclusion Technique with their solubilization rate by various T. viride cellulase preparations will be extended to other substrates and refined with respect to the precision of the analytical techniques employed. T. viride cellulase preparations and fractions will be treated on several Sephadex columns designed to separate first enzymatic components and the C_1 factor of T. viride complex by gel filtration and finally a cellobiase factor by surface adsorption. A larger quantity of the factor for physical, chemical, and enzymatic characterization will be isolated.

b. Specific Objective: To isolate and study the chemical and physical properties of hemicellulose of pith from stalks of corn, sorghum, sugar cane, and sunflowers.

Plan of Work: Holocellulose will be obtained from the pith of several species of plants. This will be separated into the cellulose and hemicellulose fractions. The chemical and physical properties of hemicellulose from different sources will be compared.

c. Specific Objective: To isolate and determine the composition and structure of the hemicelluloses in agricultural residues and to isolate a residue or fraction that is high in xylose content.

Plan of Work: The compositional differences in the hemicellulosic and fiber fractions of agricultural residues will be examined by the alditol acetate derivatives. If a suitable residue containing a high concentration of xylose is found, methods will be devised for the isolation and reduction of the xylose.

20520-012

a. Specific Objective: Continue development and evaluation of new cereal food products.

Plan of Work: Evaluation of the peanut and peanut-soy fortified CSM-type products will be completed and specifications will be prepared for the new products. Studies will be conducted on the antioxidative effects of soy flour in food blends fortified with ferrous sulfate.

b. Specific Objective: Explore stability, physical, chemical, and sensory characteristics of cereal products fortified with magnesium and zinc.

Plan of Work: Effects of adding magnesium and zinc to cereal products will be studied by determining segregation, chemical, cooking, organoleptic, and storage characteristics of the fortified products.

c. Specific Objective: Determine dry milling characteristics of different grains.

Plan of Work: Dry milling evaluations will be continued on corn preserved by the "trickle ammonia" and other processes. Corn infused with amino acids will be dry milled to determine fate of added acids.

d. Specific Objective: Investigate extrusion as a means of altering structural properties of cereals and cereal products.

Plan of Work: Study the controlled gelatinization of starch and cereal flours using extrusion and other cooking techniques.

e. Specific Objective: Extend NRRC cereal food-blend data bank to allow more flexibility in the Food for Peace program by providing standardized products from a variety of cereal and protein ingredients.

Plan of Work: Prepare and evaluate a variety of cereal (corn, wheat, sorghum)-protein (soy flour, peanut meal, cottonseed meal, nonfat dry milk, whey, and others) food blends, restricting said blends to four general end use categories, i.e., beverage, flour, gruel, and porridge. Establish a data information bank for a variety of products so that specific ingredients can be readily changed in a product, without materially altering functional or nutritional properties, to take advantage of surpluses and/or price advantages.

20520-013

a. Specific Objective: Using naturally occurring intensely sweet glycosides as a model, synthesize similar glycosides to provide a healthful sweetener.

Plan of Work: Stevioside, phyllodulcin, and osladin are naturally occurring, intensely sweet substances which will be mimicked in structure by synthesis. Synthesize nonnitrogenous glycosides of mono- and disaccharides with varying numbers of hydrophobic methylene groups, unsaturated carbon atoms, or polycyclic condensed rings between the hydrophilic groups and determine their potential for use as a noninjurious sweetener in foods.

b. Specific Objective: Provide new, sweet alditols (sugar alcohols) for use as noncariogenic and low-caloric sweeteners in foods.

Plan of Work: Prepare cellobiulose and isomaltulose by isomerization of cellobiose and isomaltose and reduce these keto-disaccharides to the corresponding alditols. Synthesize disaccharide sugar alcohols with α and β-interglycosidic linkages and compare their sweetness and digestibility to maltitol, a caloric commercial sweetener.

c. Specific Objective: Determine the toxicants and related browning reaction products that are produced upon heating sugars and amino acids in the presence of corn or other vegetable oils.

Plan of Work: The sugar-derived compounds produced by ammoniating corn and by heating sugars and amino acids in corn oil will be separated by chromatography and characterized. Their structures will be determined by spectral methods and by synthesis.

d. Specific Objective: In the column isomerization of aldoses to ketoses with aluminate resins, reduce the amount of sugar degradation that occurs at elevated temperatures.

Plan of Work: Anion-exchange columns will be prepared with additives to the aluminate solution which will be selected to reduce the hydroxide content of the resin or to increase the stability of aluminate on the resin.

20520-014

a. Specific Objective: Complete characterization of low-temperature autoxidation products of linolenate, soybean esters, and isomeric dienes from reduced linolenate, and study how the products from mixtures of unsaturated fatty esters in vegetable oils interact as precursors of off-flavors and odors.

Plan of Work: Determine hydroperoxides and identify secondary products from autoxidized linolenate, from soybean esters, from 12,15- and 9,15-dienes by the GC-MS and HPLC methods developed for autoxidized oleate and linoleate. Decompose selected fractions and determine their contribution to secondary products by preparative GLC, GC-MS, and HPLC. Synthesize and isolate autoxidation products for collaborative studies on mechanisms at University of London (see 8010-20520-247 under "B. Summary of Progress of Extramural Projects") and on nutrition at Hebrew University of Jerusalem under the auspices of the United States-Israel Binational Science Foundation.

b. Specific Objective: Prepare and evaluate polymer-supported catalysts for nutritionally desirable cis-bond forming hydrogenation.

Plan of Work: Continue work on the preparation and evaluation of improved polystyrene, polybenzoyl styrene, polyvinyl naphthalene and phosphinated polystyrene as ligands for chromium tricarbonyl complex catalysts. Evaluate catalytic activity with conjugated soybean esters with particular emphasis on recycling the catalyst. Explore conditions to minimize loss of activity on recycling. Initiate work on a two-step conjugation-hydrogenation process to achieve linolenate selectivity.

c. Specific Objective: Initiate study of mechanism of natural antioxidant action to determine how to improve flavor stability of soybean oil.

Plan of Work: Develop new information on the relative protection of tocopherols toward the oxidation of different unsaturated fatty esters. Oxidize mixtures of oleate, linoleate, and linolenate in the presence of tocopherols and determine the relative proportions of different hydroperoxides by GC-MS.

d. Specific Objective: Develop general methods for the synthesis of metabolites obtained from Cruformate that may have potential insecticidal action in animal feeds.

Plan of Work: The synthesis of 2-chloro-4-(2-hydroxy-1,1-dimethylethyl)-phenol will be investigated from readily available starting materials.

20520-015

a. Specific Objective: Determine relationship of structure and composition of proteins of wheat and related cereal grains to their functionality in bread and other foods.

Plan of Work: Isolate and separate proteins from various genotypes of wheat, hybrids of wheat, and available intergeneric crosses with wheat, such as triticale, and determine relationships of composition of protein to physical performance of flour doughs. Improve methods of isolating proteins and determining molecular weights by column chromatography and electrophoresis. Compare the N-terminal sequence of related proteins from different wheat varieties or species differing in breadmaking quality.

b. Specific Objective: Produce protein concentrates and other fractions of cereal grains having improved nutritional and functional properties.

Plan of Work: Prepare protein isolates from air-classified fractions of high protein-high lysine wheat flours. Study the modification of wheat gluten properties by the addition of various gums and lipids. Oat gums will be separated from extracts of the grain and purified and then added to cereal based diets to study the effect of the gums upon the digestive track function in humans in cooperative studies with the ARS Human Nutrition Laboratory, Grand Forks, North Dakota.

20520-017

a. Specific Objective: Expand consumer-use study of copper and nickel hydrogenated oils.

Plan of Work: Evaluate consumer use of copper-hydrogenated soybean oil in homes. Testing will be limited to two oils (nickel- versus copper-hydrogenated) and both oils will be distributed to 60 families.

b. Specific Objective: Investigate aluminum alkyls as catalysts for hydrogenation of soybean oil.

Plan of Work: Soybean oil will be hydrogenated with aluminum alkyl catalysts of the kind suggested by French workers to determine composition of products and possible utility for continuous hydrogenation.

c. Specific Objective: Conclude investigation of batch high-pressure hydrogenation of soybean oil with copper catalyst and apply results to continuous hydrogenation.

Plan of Work: High-pressure batch hydrogenations of soybean oil will be run to conclude a statistical study of effect of pressure, temperature, and copper catalyst concentration on time of hydrogenation and composition of products. This information will be applied to consideration of necessary equipment for continuous high-pressure hydrogenation.

d. Specific Objective: Investigate laboratory-scale continuous hydrogenation of soybean oil with copper catalysts to obtain and simulate on the computer kinetic information basic to larger scale continuous hydrogenation.

Plan of Work: A study will be made of preparation and activation of copper catalysts to obtain sufficient activity for the shorter residence times necessary in continuous hydrogenation. Apparatus suitable for use with the more viscous mixture of catalyst and oil produced by copper catalysts will be developed. Effect of reaction parameters on continuous hydrogenation with copper catalyst will be studied.

e. Specific Objective: To identify the qualities of an active hydrogenation catalyst and translate the requirements for making an active catalyst to a practical continuous fixed-bed hydrogenation process.

Plan of Work: Previous research has shown the necessity to optimize pretreatment of a hydrogenation catalyst in order to hydrogenate soybean oil effectively. Precise methods and techniques must be found to correlate with the pretreatment (activation) of the catalyst with a hydrogenated product having the required properties. The approach will require prereducing a catalyst with a measured amount of hydrogen followed by evaluation of the oil hydrogenated in the presence of this catalyst. After optimizing the prereduction method, the technique will be translated to a continuous column operation to develop a practical procedure and evaluate process costs.

20520-018

a. Specific Objective: Produce high-protein fractions from high-lysine sorghum flour.

Plan of Work: High-lysine sorghum flour will be air-classified into high-protein and low-protein fractions. Compositions of the air-classified fractions will be determined.

b. Specific Objective: Evaluate functional and nutritional properties of wet-milled corn germ flour in food uses.

Plan of Work: Protein efficiency ratio of wet-milled corn germ will be determined by rat assay at Wisconsin Alumni Foundation. The composition of fiber in the flour will be measured by several procedures. Nitrogen solubility, water binding, and fat retention of the germ flour will be measured. The functional and nutritive contribution of wet-milled corn germ flour will be assessed in food systems such as bread and meat patties.

c. Specific Objective: Study effect of genetic variation on the composition and properties of corn and sorghum grain proteins.

Plan of Work: Proteins of normal and high-lysine sorghums will be isolated and compared by polyacrylamide gel electrophoresis and SDS gel electrophoresis to establish a basis for nutrient differences. Zein components will be isolated by column chromatography and their amino acid sequence determined.

20520-019

a. Specific Objective: Examine the approximately 100 yeast strains isolated from samples collected in swampy areas in Louisiana during 1976 for the presence of new and useful species.

Plan of Work: Carbon assimilation and fermentation tests and microscopic observations will be performed on the strains and results compared with known yeast species.

b. Specific Objective: Clarify taxonomic position of possible new homothallic species with physiological characteristics much like the heterothallic Pichia rhodanensis.

Plan of Work: DNA will be extracted from species under consideration and compared by determining G+C content and the amount of DNA homology.

c. Specific Objective: Clarify the taxonomy of the genus Sterigmatomyces.

Plan of Work: Species will be compared by electron microscopy to determine if all bud in a manner characteristic of the type species. DNA will be extracted from all species under consideration and compared by determining G+C content and the amount of DNA homology.

d. Specific Objective: Computerize carbon assimilation and fermentation data available on cultures in the yeast collection.

Plan of Work: A computer program capable of handling the approximately 60 characters of prime interest has been constructed. Data from about 5,000 strains is to be entered.

20520-020

Specific Objective: To continue operation of the ARS Culture Collection.

Plan of Work: The staff of the ARS Culture Collection will continue its long-term operations in collecting, classifying, preliminarily evaluating, maintaining, and distributing microbial cultures for research and development involving plant residue utilization.

20520-021

a. Specific Objective: Complete construction of room odor facilities, standardize procedure for room odor evaluations, train room odor panel, and evaluate edible oils for room odor characteristics.

Plan of Work: Two identical 5 X 8 X 10 rooms will be constructed adjacent to existing taste panel facilities. Appropriate procedures for utilization of these rooms will be developed based on our previously published work on room odor evaluations. Using standard cooking oils and flavor additive procedures, the taste panel will be trained in room odor evaluation. Oils subjected to various processing conditions will be tested for effect of treatment on room odor evaluation.

b. Specific Objective: Conduct organoleptic evaluations, chemical analyses, and evaluation of processing of edible oil from soybeans sampled in the export system.

Plan of Work: Crude oil will be extracted from soybeans sampled in export shipment in sufficient quantity to facilitate complete processing and testing by sensory evaluation panels. Chemical characterization of the oils will be accomplished prior to processing to guide possible modification of procedures which may be required. Degummed oils will be processed both in batchwise laboratory procedures and continuously in the minirefinery.

c. Specific Objective: Investigate possible deleterious effects of split beans on soybean oil processing and quality.

Plan of Work: Oils extracted from soybean wholes and splits will be analyzed for iron, free fatty acid, phosphorous, and peroxide value to ascertain any differences in oil characteristics. Treatments of the whole and splits will include various bean moisture levels and simulated storage conditions.

d. Specific Objective: Evaluate conditions of steam refining to improve the removal of free fatty acids in the finished oil.

Plan of Work: Procedures for degumming soybean oil will be investigated, including (a) posttreatment with phosphoric acid following water degumming, (b) time of degumming, (c) acid concentration, and (d) temperature of degumming. Degummed oils will be steam refined, temperature and time of refining will be varied to determine the effects on removal of free fatty acids and production of quality oils. Oils produced will be evaluated organoleptically.

20520-023

a. Specific Objective: Investigate mechanism of soy phosphatidylcholine (SPC) oxidation and examine methods to inhibit oxidation in stored defatted soy flakes.

Plan of Work: Purified, unoxidized SPC will be incubated with preparations of lipoxygenase and peroxidase to determine whether oxidation and formation of bitterness occurs. Antioxidants will be incorporated into the system to determine whether lipid degradation can be prevented.

b. Specific Objective: Complete the analysis of nondigestible residue (dietary fiber) of soy products and initiate characterization of the components in the residue.

Plan of Work: Purified pepsin and pancreatin will be used under different incubating conditions to digest soy and cereal products. The amount of insoluble and soluble nondigested material will be determined and compared to dietary fiber content determined by chemical

methods. The lignins, celluloses, hemicelluloses, and lignified protein in the nondigestible residue will be characterized.

c. Specific Objective: Continue cooperative work with WRRC on longterm feeding studies with soy protein concentrate in rats. Vitamin B_{12} will be added to the casein and soy diets at different time intervals and varying levels to determine whether depletion of B_{12} levels in the body or increased B_{12} requirements cause growth inhibition.

A_2 Technological Objective: Improve the accuracy, precision, rapidity, and objectivity of the analytical methodology used to evaluate raw materials and products and control procedures.

1. Progress Report (Narrative):

20520-016

a. Specific Objective: Increase accuracy and sensitivity of mass spectral analysis of human blood lipids and analyze 1,000 or more samples.

Progress: Labeled blood lipid samples from a third human subject were analyzed. A new triple labeled technique was used without any significant loss in either sensitivity or accuracy. This triple labeled method makes it possible to compare labels to check for mass discrimination and will in the long run make it possible to run two experiments with only one feeding. Previous work has shown that the accuracy is dependent on sample size which suggests that the triple label would be only slightly less accurate than the dual labeled experiment.

Mass spectra of a large number of phenylthiohydantoin (PTH) derivatives of amino acids were run in conjunction with a project to measure the amount of soybean protein added to beef. The samples, end group amino acids obtained from sequencing beef and soy proteins, were analyzed as probe samples in the mass spectrometer. It was hoped that technology generated in the blood lipid work could be applied to the analysis of leucine, isoleucine, and norleucine, but the spectra of the three compounds were such that the computer could not find a unique solution. The work showed that the strong simple spectra of the PTH's made the system very

sensitive with a detection limit of 50 picograms. Analyses of the PTH samples showed the relative amounts of the amino acids present and that no unexpected amino acids were detectable.

b. Specific Objective: Collect and identify volatile odor compounds from trilinolenein and soybean oil by using a micro-room-GC-MS technique.

Progress: Preliminary measurements of micro-room odors from trilinolenein show fewer but more intense components than from tristearin, triolein, trilinolein, and soybean oil. Of particular importance is acrolein which occurs in the second largest amount (water is the first) and could well be the major cause of the offensive room odor formed during deep fat frying with soybean oil.

20520-017

Specific Objective: Development of analytical and separation techniques useful to products from continuous hydrogenation of soybean oil.

Progress: Separation of geometric isomers of methyl octadecadienoates has been achieved on 100-200 mesh XE-284 silver resin columns. Systems of linear equations and a method for computer solution have been developed to calculate percentage of positional octadecadienoate isomers in a mixture from the composition of alcohols, dialcohols, and alcohol esters in the ozonized and reduced diene mixture.

20520-024

Specific Objective: Adapt equipment and develop methodology for micro-scale characterization of thermal behavior and oxidative stability of pure lipids and of constituent interactions in lipid-protein-water mixtures.

Progress: Capillary-tube microdilatometry, though useful in studying thermal expansion of liquid fats, has been less than suitable for recording volume changes reversibly through the melting transition. Micromethodology for measuring oxidative stability thermogravimetrically was modified to better detect lability in waxes and solid fats.

2. Progress Reports (Achievements):

20520-016

LAGOCKI, JAMES W., EDWARD A. EMKEN, JOHN H. LAW, AND FERENC J. KEZDY. Kinetic Analysis of the Action of Soybean Lipoxygenase on Linoleic Acid. J. Biol. Chem. 251 (1976):6001-6006.

SELKE, E., W. K. ROHWEDDER, AND H. J. DUTTON. Volatile Components from Triolein Heated in Air. J. Am. Oil Chem. Soc. 54 (1977):62-67.

Other Reports:

ROHWEDDER, W. K., W. F. KWOLEK, D. J. WOLF, AND W. L. EVERHART. Analysis of a Three-Component Deuterium-Labeled Mixture by Multiple Ion-Monitoring Mass Spectrometry. Presented at the 24th Annual Conference on Mass Spectrometry and Allied Topics, San Diego, California, 1976.

20520-017

Publications:

None

Other Reports:

SCHOLFIELD, C. R. New Developments in Silver Resin Chromatography of Fatty Methyl Esters. Presented at American Oil Chemists' Society Meeting, Chicago, Illinois, September 26-29, 1976.

ELSON, C. E. Influence of Dietary Unsaturated cis and trans and Saturated Fatty Acids on Tissue Lipids of Swine. Presented at American Oil Chemists' Society Meeting, Chicago, Illinois, September 26-29, 1976.

3. Plan of Work

20520-016

a. Specific Objective: Determine accuracy and sensitivity of new chemical ionization procedures for mass spectral analysis of labeled fatty acids used in human metabolism studies.

Plan of Work: Put the new chemical ionization mass spectrometer into operation by interfacing the mass spectrometer to the PDP-12 computer and the PDP12 to the Modcomp II computer. Determine the best operating conditions for use with the chemical ionization source and run samples from human subject number 4.

b. Specific Objective: Collect and identify volatile odor components from trilinolein and extend observations on trilinolenin using a micro-room-GCMS technique to improve consumer acceptance of soybean oil in export use.

Plan of Work: Volatile odors from trilinolein, trilinolenin, and heated soybean oil will be characterized by their GC retention times, mass spectral fragmentation pattern, and psycho-physicochemical response induced in the nose of human "sniffer." Correlation of results from tristearin, triolein, trilinolein, and trilinolenein will be made with odor components of soybean oil.

c. Specific Objective: Initiate computerization of high resolution mass spectrometer to facilitate identification of unknown structures in food products.

Plan of Work: Programs will be written to collect high resolution data directly allowing accurate mass marking (to 1 millimass) of full spectra so that the output will be a table of molecular formula versus intensity rather than just mass versus intensity. If possible, the techniques necessary to do field desorption for use with phospholipids and other nonvolatile samples will also be pursued.

20520-017

Specific Objective: Development of separation and analytical techniques useful to separation of cis and trans fatty acid isomers from continuous hydrogenation of soybean oil.

Plan of Work: A study will be made of the use of silver nitrate, silicic acid in high-performance liquid chromatography to separate cis and trans fatty acid isomers; other applications of high-performance liquid chromatography related to hydrogenation work and related projects will be investigated; and other analytical procedures useful to hydrogenation will be studied.

20520-021

a. Specific Objective: Explore high-performance liquid chromatography for the isolation of volatiles formed during autoxidation of soybean oil and heating of cooking oils.

Plan of Work: Samples of methyl linolenate, methyl linoleate, and methyl oleate will be autoxidized by bubbling oxygen through the samples at temperatures above ambient. The evolved volatiles will be trapped at low temperatures and chromatographed on HPLC and GLC apparatus for separation, isolation, and identification of components. Isolated fractions collected from HPLC analyses will be evaluated organoleptically for odors. Volatiles collected during room odor evaluations will also be analyzed.

b. Specific Objective: Install, test, and operate automated analysis systems for color, pH, and fatty acid composition to monitor continuous vegetable oil processing.

Plan of Work: Commercially available sensors for color and pH will be installed or modified to facilitate installation into the minirefinery as transducers for computer monitoring of the continuous processing of oil from sound or damaged soybeans. An automated system for sampling the continuous stream and preparing the methyl esters of the triglyceride for subsequent analysis by an automated GLC will be designed, installed, tested, and utilized for monitoring vegetable oil processing.

20520-024

Specific Objective: Investigate thermal behavior and rheological properties of pure lipids and lipid-protein mixtures.

Plan of Work: Thermal behavior will be recorded by differential scanning calorimetric and thermogravimetric measurements with lipids of known structure and readily available food proteins. Similar materials will be examined by mechanical spectrometry if suitable experimental systems can be developed.

B. Summary of Progress for Extramural Projects:

1. Progress Report (Narrative):

8002-20520-034:

Of the organisms tested in bagasse, the highest amount (36%) of cellulose degraded was obtained with a culture of Chaetomium and the lowest (11%) with a Streptomyces. With beet pulp, the highest degradation was 36% with a Streptomyces and the lowest with Trichoderma (12%). With rice straw, 41% was degraded with a Penicillium sp. and the lowest (16%) with Penicillium and Trichoderma used together. The degradation of cellulose depended upon the substrate, the test organism, and their combinations. In general, the greatest cellulose degradation occurred when a single organism was used. The principal investigator estimates that over 25,000,000 tons of cellulosic wastes are available in Pakistan for conversion into single cell protein. Analyses are reported on moisture, ash, protein, lipids, fiber, lignin, cellulose, total sugars, reducing sugar, and silica, in beet pulp, bagasse, wheat, and rice straw, and rice husks, as well as analyses of the product after fermentation with the various microorganisms tested. Rice straw had the lowest protein initially, and beet pulp had the highest.

8003-20520-054

Accelerated autoxidations of crude and refined soybean, rapeseed, and linseed oils as well as Polish margarines were carried out to study the changes in tocopherols and their oxidation products. Delta- and alpha-tocopherols were more stable than gamma-tocopherol or plastochromanol and formed dimerization products. In a margarine-like model system with water present, tocopherols showed significantly decreased autoxidative properties. Characterization of the dimers and mixed dimers is in progress. Many of the dimers show reducing properties. Plastochromanol-8 and its mild oxidation products were found in these oils by column chromatography on $CaHPO_4$. Plastochromal is a more powerful antioxidant compared to alpha-tocopherol.

8003-20520-064

Preliminary investigations have been concerned with development of procedures for isolation of cellulolytic microorganisms at elevated temperatures. Some 98 thermophilic organisms were isolated from natural sources: 39 bacterial strains,

51 fungi, and 8 actinomycetes. The cellulolytic activity of these organisms was assayed against filter paper and wheat straw substrates. Thirty fungi and 10 bacteria utilized cellulose effectively. Optimum utilization of cellulose (wheat straw) occurred when the raw material was pretreated with either alkali or ammonia. Some bacteria were capable of 50% utilization of cellulose and produced 30% yield of protein.

Fermentations in 10-liter vessels with a mixture of cereal straws have been conducted with cellulolytic bacteria and fungi previous to animal feeding trials.

8001-20520-227

In 17-week experiments with laying hens, enzymic detoxified mustard seed meal (DMSM) was substituted for sesame meal, blood and fish meal and decorticated cottonseed meal in a commercial laying ration. The results showed that 100% substitution of the sesame or the cottonseed meal, or of 33% of the blood and fish meal, with DMSM gave egg production and egg quality equal to that of the controls. In these substitutions DMSM meal comprised 6% and 4% of the complete ration. Laying capacity decreased when the DMSM was fed at higher levels.

Mustard seed meal fractionated into three components gave a residue and a supernate suitable for cattle feed and a protein isolate containing 68% crude protein which was 83% of the protein in the original seed.

8001-20520-237

When sugar beet pulp was used as a carbon source, Myrothecium sp. among the four fungi tested, Paecilomyces sp., Aspergillus terreus, Myrothecium sp., and Gliocladium sp., produced the highest amount of protein, although Paecilomyces sp. yielded the most biomass. Sufficient biomass of M. verrucaria has been harvested for conducting feeding trials. Amino acid analysis indicated the E=T value of around 2.4 as compared to 3.2 of egg and 1.99 of wheat.

8001-20520-238

The investigators continued their toxicity studies on mixtures of groundnut and soybeans fermented by either Rhizopus oligosporus 2710 or 2549. Histological examination of various tissues, blood and urine analyses were carried

out. No significant differences in these values were found among the animals fed either fermented or nonfermented mixtures of groundnuts and soybeans. The protein efficiency ratios (PER) of groundnuts and groundnuts fermented by either R. oligosporus 2710 or 2549 were evaluated. There was no change in PER of groundnuts after fermentation. The average PER of ground nuts is 1.60 as compared to 2.49 of casein.

8002-20520-239

Studies were conducted on the compositional changes in sorghum grain following germination. Improvement in protein quality and ability to produce flour that produced better baked goods in mixtures with wheat flour were noted. Amino acid analyses of the sorghum flour and baked goods were conducted in collaboration with Pakistan Atomic Energy Commission, Lyallpur. Further studies were conducted to establish whether certain legume meals could replace milk or egg proteins in Pakistan-type baked goods.

8001-20520-241

Trisporic acid and beta ionone were not additive at saturation concentrations simulating carotenogenesis in Blakeslea trispora; stimulation was additive below saturation levels. Stimulations are competitive and cycloheximide sensitive indicating similarity in mode of action. Beta ionone acts at a site between conversion of 5-phosphomevalonate to dimethyl allyl pyrophosphate. Plus and minus strains contain protein that inhibits carotenogenesis by single cultures. Inhibition is lost on addition of trisporic acid to the minus culture suggesting inactivation of inhibitory protein; hence, high carotene yields in minus cultures. Mated cultures lack inhibitory protein but possess stimulating proteins. These proteins probably regulate carotenogensis in B. trispora.

8001-20520-242

Studies involved feeding soyidli to children at Sarar Village near Baroda, India, with moderate undernutrition and to children suffering from kwashiorkor and marasmus being treated at the medical college hospital. Even with very sick children, soyidli was tolerated well. In the kwashiorkor children, edema disappeared after some days of treatment and there was a weight gain from the beginning in the cases of marasmus. The kwashiorkor children, after the disappearance

of edema, also steadily gained weight. The initial feeding work indicated that the fermented product was tolerated and contributed 42% of the protein, 19% of the calories, 72% of the B_1, and 24% of the riboflavin. Six rat-feeding tests were conducted. The results of these tests showed fermented soyidli food intake was greater than the unfermented soyidli indicating increased acceptability. They also gained more weight. Intake was further increased with the incorporation of leafy vegetables and lime. Also, the weight gain per gram of protein ingested was greater with the fermented product than with the unfermented. The weight gain per gram protein of soyidli diet supplemented with greens and lime was comparable with those fed 20% casein diet. Data on rat livers are judged by protein, vitamin A, vitamin B_1, riboflavin, and niacin were better in those rats fed the fermented product than the unfermented.

8003-20520-243

Unsaturated fatty acid located in the external positions (1,3) of the triglycerides of soybean oil hydrogenated at a faster rate than those located at the internal position (2). cis,trans Isomerization was, however, greater at the internal position than at the external. A change in the reaction region from kinetic to diffusion had no effect on these observations. The use of differential thermal analysis as a method for monitoring the progress of the hydrogenation reaction of soybean oil was investigated.

8001-20520-246

Pure soybean hemagglutinin was found to enhance activity of rat intestinal lactase and beta-galactosidase. Feeding hemagglutinin to rats had no effect on their food intakes or growth rates. Forty soybean varieties and seven commercial soy flours were screened for hemagglutinating and trypsin inhibitor activities. Both activities were found in all samples although activities varied about twofold. There was no correlation between activity of the agglutinin and the inhibitor.

8010-20520-247

^{13}C NMR spectoscopy has been applied to determine quantitatively the cis/trans ratio of allylic hydroperoxides and hydroxides from oxidized methyl oleate. The 13C NMR signals of the allylic carbons bearing oxygen appear at characteristic

positions. Comparison of the peak heights of the substituted allylic ^{13}C resonances of both hydroperoxides and hydroxides gives a quantitative value of cis/trans ratios to within ca + 2% accuracy (checked by employing known mixtures of allylic hydroxy esters). The method is nondestructive and unlike conventional methods, does not require isolation of the hydroperoxides and hydroxides from crude oxidation products.

New knowledge on the stereochemistry of the primary products of autoxidation will help our understanding of the factors involved in preventing undesirable flavor development due to autoxidation in vegetable oils.

3090-20522-022-A

Plant-size prototype of laboratory air stream separator was installed at Kosbau Bros. plant at Deerwood, Minnesota, and was operated during the period of August 20 to September 14, 1976. Seven hundred and fifty thousand (750,000) pounds of green wild rice were processed through the separator at a rate of 6,000 pounds per hr. No mechanical problems occurred and the device required a minimum of attention while in operation. In a series of six tests, calculated recoveries of rice in heavy fraction ranged from 39 to 63%; this variability was undoubtedly due to lots of rice from different growers which were grown and picked under different conditions. Recovery of medium fraction rice was from 32 to 57%. In general, the light fraction rice recovery was rather low; under 5% by weight in all tests. It is believed most of the rice from this fraction would be lost in processing because of small size and low density.

Laboratory processing of the heavy and medium fractions from the six tests showed yields of grade A and B finished rice ranging from 72 to 80% from the heavy fraction with slightly more B grade in all but one test. Yields of finished rice (A and B grades) from the medium fraction was from 66 to 76% with most of the rice being of the B grade.

It appears that the air stream separator can function as an effective step of the wild rice processing operation. Removal of immature kernels and direct processing of the mature or heavy fraction should increase plant efficiency and capacity.

3090-10571-001A

Processing studies showed that the parching operation must be carefully conducted to avoid fragile kernels, white centers in the finished rice, and excessive broken rice. Parching temperatures need to be closely controlled to achieve desired results. Evaluation of flavor of wild rice from experimental and commercial samples indicate that a broad spectrum of flavor can occur, and that variations are largely due to processing and storage techniques. Maturity and storage time greatly influence the color of wild rice. Rate of water uptake of wild rice is largely a function of parching and scarification. Pigment systems of wild rice have been chemically shown to include chlorophylls, pheophytins, melanins, carotenoids, and anthocyanins. Compositional analysis of wild rice indicates presence of more protein with better amino acid balance, lower lipid content, but higher levels of linolenic acid, and about the same mineral content as most of the common cereal grains.

0703-10572-002A

Of the approximately 20 treatments studied for the effective storage of wild rice, the most economically feasible procedures appear to be ambient storage with watering and turning, and refrigerated storage. Limitations of these methods include a loss in yield of finished rice of as much as 10% after 7 days' ambient storage. Yields are generally stable for 5-7 weeks when green rice is stored under refrigeration. Because of the apparent need for a "curing" or fermentation step in the processing of wild rice, the rice must be kept wet and preservation of the seed by drying is precluded. Microbiological studies have identified a number of different bacteria and mold present on freshly harvested rice. Typical aerobic plate counts were of the order of 1.6×10^9 colonies per gram. Plate counts generally remained unchanged during the "curing" process, although there was some increase in mold count and a decrease in phychotropic bacteria. Parching of the rice naturally reduced the number of microorganisms. Commercial wild rice had plate counts ranging from 10^2 to 10^5 colonies per gram. Boiled rice, ready to eat, was essentially free of microorganisms.

2. Progress Reports (Achievements):

8003-20520-064

Publication:

SZEBIOTKO, K. AND M. PIASECKI. Isolation of Thermophilic Strains of Microorganisms and Elaboration of Simple Technological Methods for Utilization of Cellulosics for Biosynthesis of Single Cell Protein. First Technical Report, 1975-1976.

8003-20520-243

Publications:

None

Other Reports:

DROZDOWSKI, B. Effect of the Unsaturated Acyl Position in Triglycerides on the Hydrogenation Rate. Presented at the American Oil Chemists' Society meeting, Chicago, Illinois, September 26-29, 1976.

DROZDOWSKI, B. Effect of the Concentration of Some Nickel Catalyst Poisons in Oil on the Hydrogenation Course. Presented at the American Oil Chemists' Society Meeting, Chicago, Illinois, September 26-29, 1976.

0703-10572-002A

Publications:

LUND, D. B., R. HEIDEMANN, R. C. LINDSAY, C. E. JOHNSON, E. H. MARTH, AND D. A. STUIBER. Extended Storage of Wild Rice. Trans. ASAE 19 (1976):332-336.

FRANK, J. F., E. H. MARTH, R. C. LINDSAY, AND D. B. LUND. Microorganisms and Flavor Development Associated with the Wild Rice Fermentation. J. Milk Food Technol. 39 (1976):600-613.

TECHNOLOGIES FOR FOOD AND FEED USES OF ANIMALS

A. Technological Objective: Reduce costs and improve markets for animal products by product innovation, increasing processing efficiency, reducing energy requirements, better nutrient retention and improved quality, safety, and pollution control.

1. Progress Report (Narrative):

20530-001

a. Specific Objective: Test for pathogen removal in continuous cattle feedlot waste liquid (FLWL)-corn fermentation.

Progress: Fecal coliforms (FC), used as simulants for Salmonella, were killed in 12 hours in an operating continuous fermentor. The first chamber of a three-chambered fermentor was spiked with FC to give 10^9 FC/g wet weight fermenting mixture. The background count of the stored FLWL being fed (10^3-10^4 FC/ml) was reached in 12 hours; dilution by continuous feeding would cause a reduction to 3×10^8 FC/g. Death of FC is pH dependent; pH 4.3 over 36-72 hours is required to kill all FC (no FC recovered from second chamber). At pH 4.5 FC counts are reduced 100-fold over 108 hours, the discharge time of the fermentor.

b. Specific Objective: Study semicontinuous batch fermentation of fresh swine waste combined with corn.

Progress: Ten-percent portions of fresh swine waste-corn fermented product were used serially as inocula for 20 successive batch fermentations. Flasks were incubated for 48 hours at 28°C and swine waste was diluted to 10-percent solids content in this experiment. Lactobacillus populations of 10^9 organisms/g, obtained in 24 hours in the initial flask, were maintained throughout all successive transfers. Yeasts showed inconstant growth patterns during the fermentation. Variations from one to hundredfold increases (10^8 yeasts/g maximum) were obtained; but the higher levels of yeasts were not maintained through serial transfers. Instances of coliform removal (less than 100 organisms/g) were observed and correlated with high acid production (0.24-0.28 meq/g) in the flask but these optimal results were not transferable through successive flasks. A tenfold drop in fecal coliforms to 10^4-10^5 organisms/g was more typical in each flask of this semicontinuous fermentation.

c. Specific Objective: Preparation of ARS-NCR publication on the chemical and microbial composition of feedlot waste.

Progress: Chemical analyses from research contract with Denver Research Institute (DRI) and the distribution and biochemical capabilities of the aerobic microorganisms from these feedlots (plated samples received at NRRC from DRI) are included together with a more detailed microbial examination of FLW, runoff, and associated sites from an Illinois cattle feedlot. Data are included for three chemical and four microbial samples which were collected over a 1-year period from each of seven cattle and two hog feedlots; seven FLW and runoff samples and six samples from associated sites were taken over a 1-year period for microbial analysis of the Illinois feedlot. The text (40-50 pages) is one-fourth completed; 29 tables (68 pages) are 90 percent completed.

2. Progress Reports (Achievements):

20530-001

Publications:

None

Other Reports:

HESSELTINE, C. W. Solid Substrate Fermentations. Presented at Ciba-Geigy Lectures in Microbial Biochemistry, New Brunswick, New Jersey, November 10, 1976.

3. Plan of Work:

20530-001

a. Specific Objective: Test for pathogen removal in continuous FLWL-corn fermentation.

Plan of Work: Inoculate operating system with Mycobacterium paratuberculosis and follow survival by isolation on selective media.

b. Specific Objective: Study semicontinous batch fermentation of fresh swine waste combined with corn.

Plan of Work: Using standard microbial methods, examine the fermentation for reducing numbers of coliforms while maintaining a high number of lactobacilli by serially using portions of fermented product as inoculum for successive batch fermentations. Examine varied fermentation intervals for each batch.

c. Specific Objective: Increase protein-producing capacities for directed waste-grain fermentations.

Plan of Work: Continue selection and mutation studies of FLWL-corn fermentation isolates for starch hydrolysis and lysine excretion. Initiate search for cystine and/or methionine excretors. Initiate search for above isolates from swine waste-corn fermentations.

d. Specific Objective: Preparation of ARS-NCR publication on the chemical and microbial composition of feedlot waste.

Plan of Work: Complete manuscript and submit to NRRC reviewers.

TECHNOLOGIES FOR INDUSTRIAL USES OF PLANT AND ANIMAL PRODUCTS

A_1 Technological Objective: Generate new knowledge of composition, properties, processes, mechanisms, and reactions related to materials of agricultural origin, components, and derivatives as a basis for new technologies.

1. Progress Report (Narrative):

20540-003

Specific Objective: Synthesize a series of structurally different saponified polysaccharide-polyacrylonitrile graft copolymers, investigate their rheology and water absorbency, and determine by scanning electron microscopy the physical nature of the microgel particles which comprise saponified starch-polyacrylonitrile.

Progress: A series of starch-polyacrylonitrile (PAN) graft copolymers was prepared from corn starch which had been heated in water at temperatures up to 94° C to vary the extent of starch granule swelling and disruption. Graft polymerization onto gelatinized starch gave less frequent grafting of higher molecular weight PAN than comparable graft polymerizations onto ungelatinized starch. A graft copolymer was also prepared from gelatinized starch under high dilution conditions to give lower molecular weight grafted PAN and more frequent grafting. Graft copolymers were then saponified with sodium hydroxide to convert nitrile substituents to a mixture of carboxamide and sodium carboxylate. Saponified graft copolymers were only partially water soluble and consisted largely of highly swollen, insoluble gel, which was separated from solubles for the study of physical properties. Saponification mixtures were also dried to yield highly absorbent polymer films. With the exception of the graft copolymer prepared under high dilution conditions, the physical properties of saponified graft copolymers depended on whether or not the granules of starch were gelatinized before graft polymerization. Compared with saponified graft copolymers derived from ungelatinized starch, those prepared from gelatinized starch gave films which absorbed larger amounts of aqueous fluids. Also, the gel fractions from these saponified gelatinized polymers exhibited higher water swelling, lower shear modulus, and a lower reduced viscosity function (η/cQ) where c is the weight fraction of polymer and Q is swelling volume in

excess solvent of the same ionic strength. The saponified graft copolymer prepared from gelatinized starch under high dilution conditions more closely resembled those prepared from ungelatinized starch, suggesting that molecular weight of grafted PAN and the grafting frequency rather than starch granule pretreatment might be the most important factor which influences properties.

In the appropriate concentration range, saponified cellulose-PAN graft copolymer is also a viscous, closely packed, swollen gel particle suspension and thus has rheological properties similar to saponified starch-PAN. Viscosities, η, in either water or salt solution are reduced to a single master curve by use of the reduced viscosity function η/cQ. The effective molecular weight between crosslinks, M_C determined from shear modulus, corresponds to M_C values for other closely packed gel thickeners of similar η/cQ. Among all examples of this class of thickener, the plateau values of η/cQ, which occur at $cQ>2$, are approximately inversely proportional to M_C. This gel particle system has a high shear stress yield value in aqueous suspension. The yield value, i.e., shear stress extrapolated to zero shear rate, depends on reduced concentration, cQ, and rises smoothly with increasing cQ, starting at a value somewhat greater than 1. Elastic response (P_{11}-P_{22}) to oscillation depends strongly on preliminary shear treatment of the sample.

Comparison of scanning electron micrographs of a series of starch-PAN graft copolymers with those of the corresponding saponified products show that particles of saponified polymer retain the outward appearance of graft polymerized starch granules. Since starch is soluble in alkali and PAN is also rendered soluble on alkaline saponification, a crosslinking reaction either during graft polymerization or during base saponification is proposed to account for the observation that granules are not totally disrupted or dissolved.

20540-004

Specific Objective: Develop micro-scale methodology to improve characterization of potential lubricant constituents.

Progress: Freezing thermograms from differential scanning colorimetry of Limnanthes acids correlated well with solvent crystallization characteristics encountered in low-temperature fractionation of the acids. Endotherms in melting thermograms better represented the major constituent acids. These correlations promise to simplify low-temperature fractional crystallization.

Apparatus was designed for modification of a commercial mechanical spectrometer to allow study of friction and wear characteristics with less than 2 ml of finished lubricant. Initial trials indicate suitability for comparative ranking of oils.

A thermogravimetric method, developed earlier to measure oxidative stability with microliter samples of oil, required modification in order to detect lability in liquid wax esters and potential sperm oil replacements. This project was terminated on 9-30-76.

20540-008

Specific Objective: To determine the underlying basis for an apparent preferential amylolysis of the amylose component of starch by alpha-amylase bound to a phenol-formaldehyde resin.

Progress: The particulate resin carrier, Duolite S761, has been examined by scanning electron microscopy (SEM) and its sorptive character, relative to corn starch dispersions, determined. SEM revealed a structure of granular aggregrates, having interstices of submicron dimensions.

The extent and kind of polysaccharide sorption on the Duolite S761 was studied. Under conditions used to degrade starch with the enzyme-resin complex, pH 6.5 and 40° C, amylose was preferentially sorbed from a starch dispersion, whereas, amylopectin (waxy maize starch) alone was not. These results suggest the crucial role of the resin in allowing specific bound enzyme-substrate interactions. Increasing surface/volume ratio of the resin reduced the specificity of enzyme-resin reactions indicating importance of steric factors and substrate diffusion to reactive enzyme sites. Continuing investigations on the use of other carriers in treatment of paper mill effluents, e.g., chitin, have not disclosed any selective enzyme action.

An apparatus was constructed to provide rapid removal of byproducts from the reaction of a starch substrate with the bound enzyme. Oligosaccharides separated by dialysis from partially reacted substrate were in DP range 4-6 but yields per volume of effluent were very low so that absence of DP 1-3 materials needs verification.

20540-011

Specific Objective: Devise conditions that permit cells to consistently undergo nuclear changes characteristic of meiosis and isolate viable meiotic products.

Progress: Culture conditions have been identified that permit three independent stocks of C. utilis to consistently undergo nuclear changes that result in the formation of two to three nucleated refractile but nonviable bodies per cell. Nonviability has not been remedied by numerous modifications in culture conditions, although the cells containing the refractile structures remain viable and bud normally when transferred to a growth medium.

20540-014

Specific Objective: Treat the most pressing problems of crop improvement of Simmondsia (jojoba).

Progress: Each jojoba seed can be analyzed for chemical composition in less than an hour by computerized gas chromatography, and the slightly mutilated but still viable seed can be germinated if found to contain oil of desirable or unusual chemical composition. Preliminary success in sex determination of dioecious jojoba plants was attained by spectrophotometric inspection of fresh leaves ground and extracted with ethanol. This project was terminated on 9-30-76.

20540-018

a. Specific Objective: Determine reaction rates and yields when cationic starches (hydroxide form and mercaptoacetate salt) are combined with carboxylated and nonionic elastomers, respectively.

Progress: Initial attempts to produce a reprocessable starch-rubber system were made by combining one part of basic (OH form) cationic starch with three parts of a carboxy elastomer (NBR-COOH). Based on analysis of insoluble gel produced during mixing, about 60% of the total added elastomer was ionically bound to the starch. Using higher ratios of starch increased the percentage of bound elastomer to 77%. For the mercaptoacetate salt of basic cationic starch (Cato-SH), a method of analysis was developed that shows salts with up to 15 milliequivalents salt per 100 g starch are stable for 2 months or longer with no loss in activity. To study the combination of these salts with elastomers, a statistically designed series of experiments was initiated which will allow the simultaneous study of two methods of Cato-SH elastomer mixing at four starch to elastomer ratios using three different elastomers with and without a peroxide catalyst in addition to heating effects on starch-bound elastomer content. Three methods of combining starch and elastomers are being investigated to obtain maximum interaction of the two.

b. Specific Objective: Compare relative rates of formation and strengths of metal cation and ammonium carboxylate crosslinks in elastomers to determine the preferred ionic linkage for starch and elastomers and to possibly use the metal cation crosslinks to supplement starch-elastomer links.

Progress: Reaction rates of metal oxides or hydroxides with NBR-COOH decrease in the order Ba, Ca, Zn, Mg, Al, and α-(dimethyl amino)-ethylether at 175° C in an extruder and decrease in the order Pb, Ca, Ba, Zn, and Mg at 200° C in a Banbury-type mixer. Elastomer was decreasingly insolubilized by metal cation crosslinks in the same order as above (e.g., Pb, Ca, Ba had near 100% gel; Mg had 80% gel) with Ca crosslinked NBR-COOH giving excellent physical properties.

20540-020

Specific Objective: Develop techniques for plasticizing starch-based films such that the plasticizer will not be leached out of the film upon exposure to rain.

Progress: Ethylene acrylic acid copolymer was observed to serve as a plasticizer when added to a starch-film formulation and was not leached out during exposure to water. Evaluation of this new starch film is reported under Item IV A_2 1.

2. Progress Reports (Achievements):

20540-003

Publications:

BURR, R. C., G. F. FANTA, W. M. DOANE, AND C. R. RUSSELL. Graft Copolymers of Starch and Mixtures of Acrylamide and Acrylic Acid. J. Appl. Polym. Sci. 20 (1976):3201-3204.

DENNENBERG, R. J. AND T. P. ABBOTT. Rapid Analysis of Starch Graft Copolymers. J. Polym. Sci., Polym. Lett. Ed. 14 (1976):693-696.

TAYLOR, N. W. AND E. B. BAGLEY. Rheology of a Cellulose Graft Copolymer. Comparison with Other Closely Packed Gel Thickeners. J. Appl. Polym. Sci. In press.

Other Reports:

20540-004

Publications:

NIESCHLAG, H. J. A Versatile Pen-Holder for Chart Recorders. J. Chem. Ed. In press.

Other Reports:

None

20540-008

Publications:

BOUNDY, J. A., K. L. SMILEY, C. L. SWANSON, AND B. T. HOFREITER. Exoenzyme Activity of alpha-Amylase Immobilized on a Phenol-Formaldehyde Resin. Carbohydr. Res. 48 (1976):239-244.

Other Reports:

SMILEY, K. L. Enzymologic Developments. Presented at the Corn Refiners Association Conference, NRRC, Peoria, Illinois, October 19, 1976.

20540-014

Publications:

MIWA, T. K. AND G. F. SPENCER. Composition of Jojoba Oil from Nuts Harvested at Different Geographical Regions. Proceedings of the Second International Conference on Jojoba and Its Uses, February 10-12, 1976, Ensenada, Mexico. Accepted December 29, 1976.

MIWA, T. K., G. F. SPENCER, AND R. D. PLATTNER. Separation and Structure Determination of Jojoba Oil Components by High-Pressure Liquid Chromatography and Gas Chromatography/Mass Spectrometry. Proceedings of the Second International Conference on Jojoba and Its Uses, February 10-12, 1976, Ensenada, Mexico. Accepted December 29, 1976.

MIWA, T. K. AND P. H. THOMSON. Correlation Between Density and Oil Content in Jojoba Nuts Harvested at Different Geographical Regions. Proceedings of the Second International Conference on Jojoba and Its Uses, February 10-12, 1976, Ensenada, Mexico. Accepted December 29, 1976.

SPENCER, G. F., R. D. PLATTNER, AND T. K. MIWA. Jojoba Oil Analysis by High-Pressure Liquid Chromatography and Gas Chromatography/Mass Spectrometry. J. Am. Oil Chem. Soc. Accepted February 4, 1977.

Other Reports:

None

20540-018

Publications:

None

Other Reports:

ABBOTT, T. P. AND W. BROWN. Powdered Rubber. A 20-minute, 16-mm color, sound movie shown to research and development personnel, Polysar Limited, Canada, and Revertex Limited, England.

3. Plan of Work:

20540-002

a. Specific Objective: Complete studies on polysaccharide:polysaccharide interactions.

Plan of Work: Compare viscosity of various polysaccharides alone and in mixtures to determine which combinations result in enhanced viscosity or gel formation.

b. Specific Objective: Complete characterization of xanthanase and its hydrolysis products.

Plan of Work: Attempt to identify xanthan fragments in xanthanase digestions by GLC, LC, or other suitable methods.

c. Specific Objective: Complete characterization of borate complexes of mannans and phosphomannans.

Plan of Work: Study effects of temperature and pH on stability of borate complexes.

20540-003

Specific Objective: Determine water absorbency and rheological properties of a series of saponified starch-polyacrylonitrile graft copolymers to permit more efficient use of these absorbent polymers in specific applications.

Plan of Work: The series of copolymers will be prepared with grafts of similar molecular weight but differing in amount added onto the starch component. Absorbency of water and salt solutions, as well as viscosity and shear modulus, will be determined. Quantitative relationships among these properties, or others, will be sought to predict optimum polymer composition for a given application.

20540-008

Specific Objective: None. Project terminates 6-6-77 and emphasis for remaining time will be on applied aspects. (See IV A_2 3.)

20540-011

a. Specific Objective: Determine whether viability of nucleated refractile bodies in C. utilis cells is dependent upon either intracellular or extracellular fusion.

Plan of Work: Use Giemsa stain to follow the nuclear behavior of the refractile structures in order to determine whether fusion of the intracellular nuclei occurs before budding commences. Pair individual isolated refractile bodies by micromanipulation and observe them microscopically to determine whether fusion of these structures leads to the formation of a viable yeast cell.

b. Specific Objective: Begin work in conjunction with CWU 20170-002 on nitrogen fixation (see Physiological and Biochemical Technology to Improve Crop Production). Examine the sensitivity to the killing effects of ultraviolet irradiation of the blue-green alga Anabaena that is symbiotically associated with an Azolla water fern.

Plan of Work: Two independent axenic free-living algal stocks have been isolated from the Anabaena-Azolla complex. Single-cell suspensions of these isolates will be exposed to several regions of the ultraviolet spectrum (filters are available that selectively screen out parts of the UV spectrum) and their sensitivity to ultraviolet-induced kill will be examined. Survivors will be screened initially for mutations affecting pigment production, colony morphology, and growth rates. Mutants will be held as marked stocks for any future genetic studies.

20540-018

a. Specific Objective: Complete the study of nonionic elastomer reactions with mercaptoacetate salts of cationic starch to optimize the concentration of starch-elastomer ionic bonds.

Plan of Work: Perform the remaining experiments of the statistical design outlined in 1976.

b. Specific Objective: Make a tertiary amine surfaced carbon black and combine it with carboxy-elastomer to determine if the cationic carbon black, whose particle size is inherently smaller than that possible with cationic starches is more or less effective in promoting filler-elastomer reaction.

Plan of Work: React carbon black with bromine in carbon tetrachloride followed by reaction of the brominated black with dimethylamine in ethanol. The basic form (OH) and mercaptoacetate salt of the cationic carbon black will be compared to a similar form of cationic starch in several elastomers and analyzed for gel content, rheometer curing characteristics, and if possible physical properties.

20540-020

Specific Objective: Study possible routes for producing water-absorbing starch derivative that could be used in making an internally plasticized starch film.

Plan of Work: Investigate procedures for reacting epoxy polyols with starch and evaluate the properties and potential use of these starch derivatives in film applications.

20540-022

Specific Objective: Identify U.S. plant species that give promise of providing useful amounts of rubber and/or other hydrocarbons.

Plan of Work: Critically review past research to establish criteria and methods for screening plant species as domestic sources of rubber and hydrocarbons. Collect and analyze plants from several species to determine their total composition. Develop methods for characterizing the major hydrocarbon components from the more promising plants.

20540-023

a. Specific Objective: Prepare several new water-dispersible polymers representing a variety of structures that contain increasing amounts of ingredients from renewable resources.

Plan of Work: New hydroxy acids and alcohols derived from basic research on soybean oil will be utilized in the preparation of water-dispersible coatings with improved stability. Preliminary properties on films from these dispersions will be obtained to assess their industrial potential.

b. Specific Objective: Prepare soybean oil-derived solventless coatings that may furnish basic information on ways to reduce and eventually eliminate the use of both petroleum solvents and vehicles in industrial coatings.

Plan of Work: Selected new derivatives of reactive aldehyde, acetal and hydroxy derivatives will be investigated to evaluate their potential as ingredients in solventless coatings.

c. Specific Objective: Synthesize novel sulfur compounds made by the addition of hydrogen sulfide to pure conjugated fatty acids and esters for evaluation as coatings, lubricants, and plasticizers.

Plan of Work: Based on the promising lubricants obtained from nonconjugated fatty materials, investigations will continue on the addition of hydrogen sulfide to conjugated fatty esters under both free radical and ionic conditions to identify the major reaction products and to determine how the yield of specific products that may possess industrial utility can be optimized.

A_2 Technological Objective: Develop new and improved processes and products, applying known principles, which can be utilized to increase economic return to producers, reduce costs to consumers, and to satisfy recognized national and consumer needs in health and safety, product quality, environmental quality, conservation of resources, and export and balance of payments.

1. Progress Report (Narrative):

20540-002

Cationic Polysaccharides

Specific Objective: Continue to seek enzymatic or chemical methods for converting microbial polysaccharides now on hand into cationic forms.

Progress: Studies were continued on crosslinking neutral mannans with borax. Unlike phosphomannans, KCl added to mannan-borate complexes only slightly enhances viscosity and, on prolonged standing, precipitates the complexes. Incremental addition of borax to mannans increases viscosity, ultimately, to formation of clear, stable gels. Correlation of borate complex formation with mannan structure revealed that all highly branched mannans formed complexes; highly branched glucomannan, wherein all glucose occurs as nonreducing end groups, did not form a complex. Complex formation occurred with mannans of low degree of branching when unbranched α-(1T6)-linked residues were present, but not when these residues were absent.

Arthrobacter Polysaccharides

a. Specific Objective: Complete work on structure of B-1797 polysaccharide.

Progress: Polysaccharide B-1797 consists of a backbone of β-1,4-linked glucose and glucuronic acid with pyruvylated side chains.

b. Specific Objective: Complete study of viscosity improvement process for B-3225 polysaccharide.

Progress: Studies on the process were completed. The procedure which was previously developed (1976 WRU report) for increasing viscosity of native B-3225 polysaccharide was found to also work on deacetylated material. Native material so treated was found to maintain its enhanced viscosity while solubility was improved. As is the case with native polymer, viscosity of modified samples was further increased by addition of salts or acid. Potential for commercial development of this process is considerable because of the versatility of solution properties which can be obtained.

Xanthan

a. Specific Objective: Find way to maintain stock of B-1459 strains that do not change from high to low pyruvate xanthan producers.

Progress: A new substrain of Xanthomonas campestris NRRL B-1459-4L), which produces normal polysaccharide (xanthan) in high yield with both organic and inorganic nitrogen sources, was preserved by lyophilization in

sufficient quantity for future use. This substrain was obtained by selective isolation and meets all criteria for production of xanthan gum.

b. Specific Objective: Continue to study solution properties of microbial polysaccharides.

Progress: The effect of pyruvate levels on the ability of xanthan to form gels with galactomannans such as guar and locust bean gum was studied. Xanthans high in pyruvate resulted in much more gel formation with guar gum than those low in pyruvate. Conditions were determined for optimum gel formation between xanthan and various galactomannans. Other synergistic interactions were studied between various microbial polysaccharides and plant gums.

c. Specific Objective: Characterize xanthanase-producing microorganisms and xanthanase.

Progress: Two bacterial strains which physically degrade xanthan gum were identified as Bacillus sp. These microorganisms, each of which produce a different xanthanase, apparently do not have characteristics like any of the known Bacillus species. Purified xanthanase will degrade X. phaseoli polysaccharides, but at a different rate and to a greater extent than for xanthan gum, as well as low- and high-pyruvate xanthans.

d. Specific Objective: Find a suitable replacement for scarce distillers solubles.

Progress: It was found that 0.55% autolyzed brewers' yeast (Amber BYF-300) could be substituted for scarce distillers' solubles in the standard production medium for xanthan production.

e. Specific Objective: Complete studies on P-31 NMR resonances in whole yeast cells.

Progress: Resonances characteristic of mannosyl and glucosyl phosphodiesters were detected in washed cells of mucoid and nonmucoid yeasts from the genus Hansenula and related genera. These resonances were also observed in cell-wall preparations from these yeasts and in

mannans extracted from the walls. The resonances were correlated with sugars released by mild acid hydrolysis from the extracted mannans. P-31 NMR demonstrated that cell-wall phosphomannan was conserved in only one of four phosphomannan-producing yeasts grown under phosphate limitation.

20540-003

a. Specific Objective: Investigate the feasibility of producing saponified starch-polyacrylonitrile (Super Slurper) in a continuous process, investigate processing variables in pilot plant syntheses, and determine how minor amounts of other monomers, grafted along with acrylonitrile, influence the properties of Super Slurper.

Progress: There has been a sustained high interest in the saponified starch-polyacrylonitrile graft copolymer called Super Slurper. About 1,000 additional inquiries from industrial, university, and ARS scientists have been answered with technical data and samples. Five more licenses have been granted by USDA to make, use, or sell Super Slurper; and two more companies have listed with us as suppliers of developmental quantities of the polymer. In February of 1977, the inventors of Super Slurper received the Inventor of the Year Award for their U.S. Patent 3,997,484 (one of 70,000 patents issued in 1976) from the Association for the Advancement of Inventions and Innovations. The greatest interest in Super Slurper continues to be for agricultural applications, and the currently limited production of the polymer appears to be the chief detriment to its widespread use. Of particular interest is the aerial seeding in the spring, 1976, of about 3,000 acres of Super Slurper-coated soybeans into standing wheat (double cropping). In spite of the dry weather, the company performing the seeding test was sufficiently encouraged by the results to make plans for expanding their acreage in 1977. Our pilot plant syntheses of Super Slurper have been scaled up to produce over 100 lb of dry polymer in each reaction, with good reproducibility.

Polymers with good absorbency were obtained from a variety of different starches, such as corn, waxy corn, wheat, and potato, and either sodium or potassium hydroxide may be used interchangeably for saponification. Polymers contain no detectable acrylonitrile and less than 10 ppm of acrylamide. Although a lower absorbency final product was obtained when the acrylonitrile:anhydroglucose unit mole ratio in the graft copolymerization was reduced from 3.3 to 1.65, absorbency was not increased when the higher ratio of 6.6 was used. A graft copolymer composed of about equal weights of starch and PAN is, therefore, about optimum. Comparable absorbencies were obtained when reaction products were neutralized with either nitric, acetic, or phosphoric acid during alcohol precipitation; however, use of sulfuric acid produced a lower absorbency product due to unextractable sulfate salts. Although microorganism growth in undried reaction mixtures was rapid at a near-neutral pH, mixtures could be stored for extended periods before drying at an alkaline pH. Revised and updated cost-to-make estimates for a crude drum-dried Super Slurper and a refined, alcohol precipitated product were 32 cents and 45 cents per pound, respectively, for a plant producing 10 million pounds per year annually.

Polyacrylonitrile-containing graft copolymers of corn starch were prepared from monomer mixtures in which selected comonomers were substituted for a portion of the acrylonitrile. Graft copolymers were then saponified and the saponified polymers dried to form absorbents for aqueous fluids. Use of 2-acrylamido-2-methylpropanesulfonic acid as a comonomer resulted in shorter saponification times and more absorbent products (up to 5,000 g water per g polymer). Incorporation of acrylamide into the polymerization mixture shortened the time needed for saponification to 10-15 min thus making a continuous process practical. Vinylsulfonic acid, acrylic acid, methyl acrylate, methyl methacrylate, and styrene were also used as comonomers, and their effect on saponification time and on absorbency of the saponified polymer was examined. Substitution of flour or corn meal for starch in the ceric-initiated graft polymerization of acrylonitrile gave polymers which, after saponification, had a higher absorbency for aqueous fluids than saponified starch-gPAN.

b. Specific Objective: Prepare latexes by graft copolymerization of acrylonitrile-styrene and acrylonitrile-isoprene mixtures onto cationic starch and evaluate the products for their utility as reinforced rubber and as carriers for pesticides.

Progress: Graft copolymerization of isoprene (IP) and acrylonitrile (AN) onto gelatinized starch and cationic starch having quaternary amine functionality through cerium(IV) initiation gave grafted side chains of poly(IP-co-AN). Grafts of various compositions are obtained by controlling the amounts and ratios of monomers added to starch. IP alone does not homograft onto gelatinized starch at 25° C or 50° C by cerium(IV) initiation and requires the presence of an "initiator-monomer" such as AN to obtain copolymer side chains. Starch graft reaction mixtures were sonified at 20 KHz to form latexes that air dry to clear pliable films. Poly(IP-co-AN) obtained by acid hydrolysis of the starch portion of the grafts failed to dissolve in either dimethylformamide or benzene thus indicating presence of crosslinks. Starch-g-poly(IP-co-AN) having about one-third starch and grafted side chains averaging about 2 parts polymerized IP per part of polymerized AN, was masticated on steel rolls at 100° C to a tough pliable film which was subsequently vulcanized to a rubber.

The temperature, ratio of anhydroglucose units to cerium(IV), and the initial molar ratio of monomers (0.663) for cografting styrene (STY) and AN onto starch were selected from the study on cografting IP and AN onto starch. The initial molar ratio of 0.663 was that found to give the greatest weight yield of grafted products having the highest molar ratio of polymerized IP to AN moieties. Starch-g-poly(STY-co-AN) products were prepared which contained up to about 30% add-on.

Selected latexes were evaluated in preliminary tests as suspending media for pesticides in sprayable applications. Results indicate that the volatility of certain pesticides is reduced when latex-pesticide mixtures are sprayed onto soil surfaces and allowed to dry.

20540-004

Specific Objective: Complete preparation and evaluation of candidate extreme pressure lubricants and sperm whale oil replacements.

Progress: Eleven additional quarts of candidate fluids were submitted for sulfurization and evaluation. The chemical conversion of crambe acids to wax esters was simplified, but with some loss of lubricating properties and thermal stability. Wax esters made chemically from monoene-enriched Limnanthes acids were comparable to sperm oil in lubricating properties and better in thermal stability. Crambe wax esters and liquid wax esters from jojoba were comparable to or better than sperm oil in simulated in-use tests of differential gear, engine crankcase, and automatic transmission lubrication. The jojoba wax esters performed better overall, but the crambe wax esters improved crankcase oil sufficiently to pass published test standards. This project was terminated on 9-29-76.

20540-005

Specific Objective: Continue cooperative field and laboratory studies on linseed emulsions; continue advising on their manufacture; and generate additional interest in their one-step application for curing and protecting concrete.

Progress: Conversations with manufacturers of linseed oil emulsions (LSOE) indicate that interest in using the emulsion is high among State Highway Departments even though there has been little publicity concerning this project over the last year. Other government agencies including the Navy Department and General Services Administration have expressed a definite interest in this development and have submitted their revised specifications on treating portland cement concrete to NRRC scientists for their review and to suggest where LSOE could be used to advantage on projects carried out by these government departments. Suitable revisions in these specifications have been made and returned to the agencies concerned. The State of Iowa conducted tests with LSOE for curing and protecting concrete on experimental sections of highway and bridge decks last year and reported that they were entirely satisfied with its performance. Iowa plans to use LSOE more extensively on projects in the Summer, 1977. This project was terminated on 9-30-76.

20540-006

a. Specific Objective: Develop premixes of high starch content that can be blended with rubber to achieve reinforcement comparable to that obtained with carbon blacks.

Progress: In a detailed statistical study, coprecipitates were prepared containing 23 to 90% starch xanthide (SX), extender oil, and rubber. These coprecipitates were dehydrated and the oil was extracted with alcohols or acetone. Good reinforcing products were only obtained when starch contents did not exceed about 45%. Some dry powdered preparations with 70 to 90% SX would disperse well in rubber by direct milling. However, these products were less reinforcing than either carbon black or SX incorporated by the usual masterbatching procedure.

Conventional and SX reinforced rubbers from several molding compounds and a tire tread compound were compared during 90 days' storage at various controlled humidities and during 1 year's outdoor weathering. These studies indicate that all the silica and part of the carbon in conventional rubber formulations can be replaced with SX without adversely affecting the humid age stability and weatherability of molded rubber goods.

The project was terminated on January 17, 1977.

b. Specific Objective: Study plant sources of rubber and other hydrocarbons.

Progress: Since the U.S. is dependent on foreign sources for natural rubber (NR) and because green-plant production of NR and other hydrocarbons has been proposed as an energy resource and conservation measure, a critical review of past research on U.S. rubber-bearing plants and screening of plants for NR and other hydrocarbons was begun. Because of encouraging results during these preliminary studies, a new CRIS work unit on this subject has been submitted and approved.

20540-007

a. Specific Objective: Improve release properties of starch-encapsulated pesticide formulations and cooperate with interested scientists in evaluation of the formulations in bioassay tests.

Progress: The technique devised to encapsulate pesticides in a crosslinked starch xanthate matrix has been improved by introducing various modifications. Crosslinking of the xanthate can be accomplished with oxidants, polyvalent metal ions, or difunctional reagents such as epichlorohydrin. Flour can be substituted for starch, and the protein component may provide different release properties of active agent from the resulting matrix. Use of a partially degraded starch or flour for xanthation and subsequent crosslinking allows a much higher concentration of xanthate to be employed. With a highly modified flour, concentrations of xanthate up to nearly 60% are workable in the encapsulation process. Of course, this has definite economic advantages since much less water has to be removed from the encapsulated product than when a starch xanthate of only 15% concentration is employed. However, as concentration of xanthate increases, amount of pesticide which can be encapsulated decreases. About a 15% active ingredient product can be prepared when a 50% starch xanthate is employed.

Release properties of the encapsulated products can be varied by incorporating other polymers into the viscous dispersion prior to crosslinking. Polymers such as polystyrene, polyethylene, and poly(vinyl chloride) can be first dissolved in a solvent such as acetone or benzene and added to the dispersion. When the xanthate is crosslinked, the polymer precipitates and becomes entrapped along with the pesticide. Control of the amount and type of polymer added can influence properties of the final encapsulated product.

At the request of numerous ARS, university, and industrial scientists, we prepared over 200 encapsulated samples from 63 different pesticides. Samples of the encapsulated products were provided to the cooperating scientists for laboratory and greenhouse bioassay tests. In some instances, the results were so encouraging that field

tests were made. An ARS cooperator at Purdue University found in replicated field tests that the starch encapsulated products were two to three times more effective in controlling weeds than the same level of the unencapsulated commercial herbicide. One application of the starch-based formulation, when applied at the highest allowable rate of application, controlled all weed growth during the entire growing season. The commercial product only controlled weeds for about 50 days.

One commercial firm has been granted a license from USDA to practice the technology covered in our patent application. Several other firms are evaluating the technology with their own particular pesticides and have reported good results. At least three companies have told us they plan to seek a USDA license to use our technology.

b. Specific Objective: Evaluate various polymeric systems for incorporation of pesticides, which upon application to foliage, soil, or seed, provides a protective film to reduce loss of pesticide due to volatility and leaching.

Progress: Samples of the methanol-treated activated sludge polymer (MAS) and dialdehyde starch (DAS) were sent to several interested cooperating scientists for their formulating with pesticides and evaluation. We also prepared formulations containing pesticides and provided these to interested scientists. In field tests with the MAS-DAS composition containing a herbicide, excellent control of weeds was obtained by a surface application. The commercial pesticide applied as recommended by the producer gave poor control. The MAS-DAS pesticide formulation was also prepared in pellet form for application as a granular product.

A starch graft polymer latex was evaluated as a polymeric system for applying pesticides and preliminary results from a surface application show it to be effective in reducing losses of the pesticide.

c. Specific Objective: Prepare a series of starch compounds containing covalently bonded pesticides through ester and acetal linkage for rate of release testing and explore other types of linkages for attaching pesticides to starch.

Progress: Starch esters of 2,4-D containing 30-50% active agent were prepared from pregelatinized, cyanoethyl and pearl starch and the products were evaluated in soil tests for toxicity towards germinating lettuce seeds. Levels of active ingredient from 2.5 to 10 pounds per acre were employed. At these levels some toxicity toward lettuce was noted especially for the products based on pregelatinized and cyanoethyl starch. Results indicated that for best performance of these types of products, a small amount of the free pesticide should be blended in with the covalently bonded material to provide immediate control of weeds.

Since several herbicides have carbamate, urea, or amine types of groups, attempts were made to join these to starch through covalent linkages. Reaction of formaldehyde with the nitrogen in such groups can lead to methylol derivatives which are reactive toward hydroxyl groups resulting in a methylene acetal bridge between the pesticide and starch. Methylol derivatives of urea types could not be successfully formed when an aromatic ring was attached to the nitrogen. Urea itself did form a dimethylol derivative and it did react with starch to give the expected product. Amiben, a herbicide which has an amine group attached to a benzene ring, condensed with formaldehyde to give the methylol but it would not then condense with starch. Attempts to form Schiff base linkages between dialdehyde starch and herbicides containing primary or secondary amine groups were not completely successful.

Attachment of 2,4-D to a simple sugar was accomplished through a mixed carbonic anhydride [R-O-C(O)-O-C(O)-O-R'] type of linkage. Preliminary tests showed this linkage to be more readily hydrolyzed than an ester linkage.

20540-008

a. Specific Objective: To evaluate in pilot plant scale trials the efficacy of paper mill effluent treatments using bound enzyme-based systems that are economically feasible and, thereby, conclude this project.

Progress: A continuous-stirred tank reactor was designed and built. Wastewater from a local mill was obtained but needed to be modified by addition of starch and clay for meaningful evaluation. Several technical problems of the process as designed were uncovered during the work. Line settling of white-water solids, sensitivity of results to pipe sizes and screening necessary to retain the bound enzyme, deterioration of the commercial white water during storage and transportation and nonlinear response in the alum flocculation stage turned out to be unexpectedly critical factors in the pilot plant operation. It was concluded that while the process could be made feasible, the time remaining would not permit the redesign of the reactor as indicated from the results obtained to date.

b. Specific Objective: Study properties of bound alpha-amylase.

Progress: In the course of this work, an apparent preferential amylolysis for the amylose component of starch by alpha-amylase bound onto the phenol-formaldehyde resin was noted. Experiments were then performed to determine limits of the hydrolysis and to characterize the polymeric and oligosaccharide products. Dispersions, up to 10% concentration, of corn starch were modified by this process and the products, obtained in yield up to 93%, had little or no loss of intrinsic viscosity but did have greatly improved resistance to solution retrogradation.

20540-009

a. Specific Objective: To determine effects of the newly completed, closed, white-water system of the NRRC pilot paper machine with respect to material balances, machine operability, and paper quality.

Progress: Various subsystems of the closed white-water loop have been evaluated in the course of continued work on the NRRC pilot paper machine relative to development of cereal-derived papermaking adjuvants. Refinements were made on the low-consistency cleaners through provision of a new pump of higher capacity and pressure. Also, the piping was modified to achieve a dynamic balancing within the cleaner system.

b. Specific Objective: Development of cereal products as papermaking adjuvants.

Progress: Certain work on development of cereal products as papermaking adjuvants was completed on the NRRC pilot paper machine. Surface sizing with cationic flours and starches, including commercial control materials were completed. Cooperative work under a Memorandum of Understanding was begun with an industrial manufacturer of paper related to use of carbamoylethyl starch as a wet- and dry-strength agent in alkaline systems. Trials on the NRRC paper machine were made using furnishes from the cooperator. A rotary sheeter was received and put into service, requiring design and construction of a collector (sheet stacker).

20540-010

a. Specific Objective: To further modify standard laboratory handsheet equipment and forming procedures to reduce time required for attainment of a high degree of process water reuse and to determine in such a system the effects on performance of cereal-derived papermaking adjuvants.

Progress: A new handsheet forming system was devised that simulated process water recycling and which attains a high level of reuse but with reduction in sheet weight from 360 to 90 gsm. The basis weight reduction eliminates excessive physical entrapment (screening out) of fines that otherwise might invalidate results. Characteristics of paper made in this system, with and without fillers, were determined and compared to results when a cereal-based additive, cationic starch, was used. The deterioration of the effectiveness of this widely used starch derivative could be correlated with extent of water reuse and dissolved solids buildup. This information will be directly applicable to setting up trial conditions on the NRRC paper machine in evaluation of additives in closed white-water systems. This project was terminated 10-19-76.

b. Specific Objective: Evaluate starch derivatives in papermaking.

Progress: Concurrent with this project there has been continuing service work and cooperative applications research with potential papermaking chemicals derived from cereals in related projects in the CPD Laboratory,

NRRC. Starch graft latices as paper surface sizes gave good dry-strength improvements, without reducing tearing strength. Starch polyampholytes, containing various ratios of cationic to anionic substituents, were prepared and evaluated. Contrary to expectations, based on xanthate heteroderivatives, imparting of wet strength to paper is not a necessary consequence of heteroderivatization of starch. Comprehensive testing (wet stiffness) of paper and paperboards made at NRRC over the last 8 years was undertaken and completed as a preliminary step in cooperative work with the ARS Agricultural Marketing Research Institute. Results which identified effective adjuvants, will guide the structuring of any further joint work on development of improved packaging material.

20540-012

a. Specific Objective: Continue work on preparation and evaluation of new water-dispersible polymers from linseed and soybean oil.

Progress: Water-dispersible urethane polyesteramide coatings from soybean and linseed oils were characterized by fair to good storage stability, rapid drying, and good hardness and solvent resistance. NRRC products dried faster to yield harder films which had excellent xylene resistance but were poorer in Spic'n'Span resistance compared to two commercial water dispersible resins. On standing, the pH of the dispersions dropped from 8 to 6.0 and the polymers precipitated slowly. Acid values of these precipitated resins revealed the loss of carboxyl content, half their original value in some. Work is in progress to design new polymer compositions with improved stability of their aqueous dispersions. These products contain reduced amounts of petrochemical solvents compared to traditional linseed and soybean coatings, and they save energy in processing and application.

b. Specific Objective: Investigate addition of hydrogen sulfide to polyunsaturated linseed and soybean oils for the preparation of lubricants.

Progress: The basic research on the chemistry of the reaction of hydrogen sulfide with nonconjugated polyunsaturated fatty acids from linseed and soybean oils has been worked out and the major products identified. With this knowledge, new sulfurized lubricants based on these oils have been prepared. In cooperative work with the ARS Eastern

Regional Research Center preliminary evaluation of selected products as antiwear and extreme pressure lubricants indicates that they outperform sperm oil derivatives. Studies on the reaction products from hydrogen sulfide and conjugated fatty acids from soybean oil are now in progress and the reaction appears to take a significantly different course.

c. Specific Objective: Continue studies on the cause and inhibition of the yellowing of linseed oil.

Progress: Films prepared from several "synthetic linseed oils" demonstated that the yellowing tendency could be attributed to one component. Films that yellow in the dark bleach rapidly in sunlight but re-yellow in the dark; a cycle that can be repeated at least 3 times. Low molecular weight aldehydes and optical brighteners reduce yellowing but metallic driers may intensify it.

This project was terminated on September 30, 1976. Similar work on soybean oil is being initiated under CRIS Work Unit 20540-023.

20540-013

Specific Objective: Examine microorganisms that can produce from grain-derived substrates useful amounts of enzymes therapeutically effective against neoplasms; study properties of enzymes; and develop economic methods for their production, isolation, purification, and stabilization.

Progress: The enzyme, L-serine dehydratase, from Aeromonas punctata NRRL B-928 was found to be inducible. Original yields of 0.07 IU/ml were increased over 50-fold to 4 IU/ml with the addition of beef extract and L-serine to the growth medium. Sonic disruption liberated the enzyme from the cells quantitatively. Purification techniques such as salt precipitation, heat treatment, and column chromatography produced preparations with activities to 300 IU/ml. Molecular weight of this enzyme was estimated to be 80,000 by molecular sieve chromatography.

A bacterium, as yet unidentified, has been selected out of a number of bacteria isolated from various sources by enrichment techniques. A yield of 0.8 IU L-serine dehydratase/ml has been attained with this organism. The enzyme does not appear inducible with L-serine, but media changes and other improved conditions might augment the yield.

20540-014

Specific Objective: Improve oil quality through development of specific refining methods.

Progress: Refinement by heat treatment did not change the thermal conductivity of jojoba oil, thus eliminating the possibility of viscosity increase and foaming at high temperatures when used as transformer oil. Jojoba oil, refined by heat treatment and filtration, performed very well as a sulfurized extreme-pressure lubricant. Its simulated in-use performances for gear, transmission, and engine tests equaled or surpassed those of sulfurized sperm whale oil, and the overall research evaluation ranked it as a top candidate for sperm whale oil replacement lubricant.

Physical properties of the oil and hydrogenated derivative were determined with emphasis on blends with hydrocarbon polymers. Hydrogenated jojoba wax and low-density polyethylene have essentially identical crystallographic unit cell dimensions, and synergism causes mixtures of the two to be nearly twice as hard as either solid alone. A new method with redefinition of wax surface hardness-testing was developed for comparing waxes of widely differing hardness. This project was terminated on 9-30-76.

20540-015

a. Specific Objective: Study mildew and blister resistance of solventless and water-based paints from linseed oil and from treated pigments.

Progress: Surface properties of a rutile titanium dioxide pigment were studied by measuring sedimentation volumes in a series of liquids of differing solubiltiy parameters. Results show the sedimentation volumes of the rutile to be most affected by the hydrogen bonding solubility parameter of the liquid. For the series, the minimum volume occurred at a hydrogen bonding parameter of about 7 and is an indication of the nature of the rutile surface. Mildewed test panels have been indexed, photographed, repainted, and placed again on our test fence. From this experiment we hope to learn how mildew reoccurs after being painted over.

b. Specific Objective: Initiate studies on the feasibility of using vegetable oil emulsions as carriers for pesticides to replace currently used petroleum derived materials and to prevent environmental contamination.

Progress: Ability of several processed linseed oils to act as solvents was investigated by determining their miscibilities with each liquid in a selected series. While linseed oils are generally nonpolar in nature, they are miscible with many polar liquids. The precise miscibility range appears to depend on the individual linseed oil. Values found in the literature for static contact angles of water on plant leaves vary from about 40 to 170. Much of the variation in contact angle appears to depend on the configuration of the particular plant surface.

Information gained so far about solvent properties of linseed oil and about contact angles of liquids on leaves and stems of plants shows a strong possibility for replacing petroleum derived materials with vegetable oils in some pesticide applications. This project was terminated September 30, 1976.

20540-016

Specific Objective: Prepare and evaluate plasticizers and lubricants from vegetable oil derivatives.

Progress: Fourteen derivatives of 9,9(10,10)-bis(hydroxymethyl)-octadecanoic acid were prepared and characterized. These included acetyl and acetone acetal derivatives of various alkyl bis(hydroxymethyl)octadecanoates, which were evaluated as plasticizers and lubricants at ERRC. The acetyl esters showed good compatibility for poly(vinyl chloride) (PVC). Comparison with the 12-acetoxy and 12-acetoxymethyl esters previously evaluated showed the bis(acetoxymethyl)octadecanoates to have better compatibility, generally higher tensile strength, much lower migration and volatility losses, and intermediate heat stability. 2,2-Dimethylpentyl 9,9(10,10)-bis(acetoxymethyl)octadecanoate was prepared and is being evaluated as a lubricant at the Naval Research Laboratory. Bis(hydroxymethyl)-octadecanoic acid is being evaluated as a component of both solventless and water-based coatings under WRU 20540-023. This project was terminated 9-30-76.

20540-017

a. Specific Objective: Preparation of cationic cereal flours will be scaled-up to provide multipound quantities sufficient for evaluation as papermaking adjuvants on the NRRC pilot paper machine.

Progress: Quatenary amine flour and starch derivatives have been prepared successfully in sufficient multipound quantities, by optimized dry state reaction conditions, to permit evaluations as wet- and dry-end additives on the pilot paper machine. Dry mixtures of starches were treated with 8-10 weight percent of 3-chloro-2-hydroxypropyl-trimethylammonium chloride and alkali catalyst for either 3 days at 25° C or 6 hr at 60° C. Cationic efficiency, streaming current values, and handsheet tests show the products to be of high quality. Interest by both cereal product and paper manufacturers in our cationic products, because of the minimized pollution in manufacturing, is expected to lead to cooperative work agreements.

b. Specific Objective: Conduct studies on evaluation of cationic, anionic, and amphoteric flour and starch derivatives as textile sizes.

Progress: A laboratory model slasher and a laboratory shed tester have been fabricated. Starch acrylic acid graft copolymers, quaternary cationic starch derivatives, and some amphoteric starch derivatives were compared as textile sizing agents with commercial samples of PVA, CMC, and modified starches. The sizing agents have been applied to yarns of cotton, and cotton-polyester blends using the laboratory slasher. Yarn sized with the commercial sizing agents and with the experimental derivatives were evaluated as to breaking strength and elongation on the Instron tester, as to shedding on the shed tester and were examined microscopically for fiber lay and sizing uniformity. Preliminary results indicated that some of the experimental derivatives are better sizing agents than the commercial starches but inferior to PVA and CMC.

c. Specific Objective: Prepare cereal-based graft copolymers by dry state reaction conditions.

Progress: Preliminary studies indicated that cationic and anionic graft copolymers of flours and starches can be prepared by dry state reaction techniques. Dry mixtures of flours or starches are treated with either 2-hydroxy-3-methacryloyloxypropyltrimethylammonium chloride (I) or 2-acrylamido-2-methylpropane sulfonic acid (II) for 2 hr

at 100° C. With preparation of the cationic derivatives of I, small amounts of hydrogen peroxide and ferrous salts are dry blended into the mixtures before heating. In the case of the anionic derivatives of II, a small amount of alkali is dry blended into the product for neutralization. Cationic efficiencies and streaming current measurements (screening tests) show them to be quality products.

20540-018

Specific Objective: To obtain ionically crosslinked starch-based elastomers that may be useful for making reprocessable rubber and scale up production of promising products to the pilot plant stage.

Progress: An efficient method for preparing 20 pounds or more of Cato 14 (basic form) was developed in the pilot plant. Also, pilot plant trials on a small particle size spray-dried starch were initiated.

20540-019

a. Specific Objective: Prepare and evaluate water-soluble starch based polymers as flocculating agents and water-insoluble starch-based products as heavy metal removing agents.

Progress: Several water-soluble starch-based flocculants were prepared and evaluated in flocculating metal hydroxide suspensions with negligible success, apparently because their molecular weights were too low. Water-insoluble starch based products have been prepared which show promise as heavy metal scavengers. Starches containing cationic single unit side chains appear to be more effective than long grafted cationic chains. Several of these products have been supplied to interested industrial personnel, and they are now being evaluated in on-site tests.

b. Specific Objective: Prepare starch products containing anionic groups other than carboxyl and complete study of the influence of reaction variables on starch product yield and performance.

Progress: Starch-based products containing sulfonic acid groups have been prepared, and preliminary evaluations have been conducted. Several of the products were made from a starch dextrin, and these were ineffective as

flocculating agents. Products with higher molecular weights have been prepared, and these will be evaluated. Reaction parameters in preparation of various derivatives were studied and optimum reaction conditions were established.

20540-020

a. Specific Objective: Develop, from a starch-derived product, flame-retardant polyurethane foams having industrial potential.

Progress: Because of industrial interest in halogenated allyl glucoside polyethers prepared earlier from glucose for making flame-retardant urethane foams, a more industrially attractive procedure was investigated for making these polyethers. In the alternate route, a commercial-grade methyl glucoside polyether was first reacted with allyl alcohol and subsequently with bromine. Preliminary evaluation of foams made from the new polyethers suggests that the foams have physical properties and flame-resistance acceptable for many building requirements as insulation. Preliminary results were encouraging in efforts directed to produce raw materials for making flame-retardant flexible foams that could be used as cushioning materials in furniture, automobiles, and the like. Sorbitol was reacted with 3 to 4 moles of allyl halide, 50 moles of propylene oxide, and then with bromine. Additional efforts are needed to increase the rate of propoxylation and the percentage of reacted bromine.

b. Specific Objective: Develop techniques for combining starch with various commercial resins to obtain biodegradable films for mulching and packaging applications.

Progress: Various polyethylene resins were combined with starch and cast or molded into thin films. The most successful resin evaluated was a commercial product obtained by copolymerizing 20 parts of acrylic acid with 80 parts of ethylene (EAA). Uniform films were obtained from combinations of starch and either the ammonium salt solutions of EAA or an EAA latex. In the presence of a small amount of stearic acid, the EAA ammonium solution could be formulated at higher solids concentrations and in general, appeared to be the more promising of the two systems for starch-film production. Tensile strengths of the films ranged from 2,400 psi with 10% starch to 7,200 psi with 90% starch. As the level of starch was increased, the films became less flexible as evidenced by decreasing percentage elongation and fold endurance. Films containing

up to 80% starch were judged to have sufficient strength and flexibility for a variety of mulching and packaging applications. When exposed to 13 cycles of 24 hr water soaking and 24 hr drying, those films with 10 to 40% starch remained very flexible and strong, those with 50 to 70% starch remained in fair condition but showed some loss of strength and flexibility while those with 80 to 90% starch became brittle. Generally, starch films are destroyed after a few minutes of water soaking; hence, the EAA greatly improved the water resistance of these starch-based films. Good water resistance is especially important for mulching applications.

Starch and EAA were also formed into quality films by dry mixing on a rubber mill or by coprecipitating starch xanthide and the EAA from aqueous dispersions then drying the precipitate and fluxing it on a rubber mill. Either method yielded films with sufficient strength and flexibility for a variety of applications; however, lower levels of starch (up to about 50%) must be used to produce quality films than is possible by the casting technique.

As another approach, a variety of starch graft poly(methyl acrylate) copolymers, having grafted chains with molecular weights of less than 500,000, were developed that were easily extruded into films with good mechanical properties. The films showed excellent susceptibility to fungal growth, some samples losing more than 40% of their weight after 22 days incubation with Aspergillus niger. Tensile tests and scanning electron micrographs of the incubated samples indicated substantial biodegradation of the copolymer. These materials may have application as biodegradable plastic mulch.

c. Specific Objective: Prepare a starch film, using starch as the sole polymer, which may have application as packaging material for agricultural chemicals.

Progress: Corn starch was modified with varying amounts of lactic acid by heating well-blended mixtures of the two components at 95 to 98° C for 1 hr. Modification in this manner produced esterification up to D.S. 0.12, probable complex formation between the amylose fraction and lactic acid and some degradation of the starch. Film prepared by casting 20% aqueous dispersions of the modified starch, using glycerol plasticizer, had tensile strengths of 1,856 psi after 7 days aging and 3,646 psi after 28 days. An elongation of 24% and a flexibility of 78 double folds, each measured after 7 days aging, were significantly greater than corresponding values for

similarly plasticized films derived from unmodified and commercially acid-modified corn starches. Normally films made from corn starch, without any additional polymer, are very brittle upon drying. Hence, this new method may provide a technique for increasing the level of starch in biodegradable films.

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20540-002

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20540-007

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Other Reports:

ANONYMOUS. Natural Polymers Control Pesticide Release. Chem. Eng. News., June 1976.

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20540-008

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SMILEY, K. L., B. T. HOFREITER, J. A. BOUNDY, AND S. P. ROGOVIN. Paper Mill Effluent Clarified with Immobilized alpha-Amylase. In Proc. 3d Biodegradation Symp., Kingston, Rhode Island, August 17-23, 1975, Sect. XV, pp. 1001-1011 (1976).

20540-009

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HAMERSTRAND, G. E., H. E. SMITH, S. H. GORDON, M. I. SCHULTE, AND C. R. RUSSELL. Carbamoylethyl Starch as a Wet- and Dry-Strength Agent. Experiments with Kraft Bag Paper. Tappi 60 (1977):131-133.

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CARR, M. E., B. T. HOFREITER, AND C. R. RUSSELL. Starch Polyampholytes with Amine and Xanthate Substituents. J. Appl. Polym. Sci. In press.

HEATH, H. D., B. T. HOFREITER, P. J. BORCHERT, M. I. SCHULTE, J. L. NEFF, AND C. R. RUSSELL. Dialdehyde Starch Hydrazones: Preparation and Properties of Cationic Dispersions. Staerke 28 (1976):303-308.

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Other Reports:

CARR, M. E. A Starch Polyampholyte for Paper. Presented at Annual TAPPI Meeting, Atlanta, Georgia, February 14-16, 1977.

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Publications:

BELL, E. W., J. C. COWAN, AND L. E. GAST. Sperm Oil Replacements from Selectively Hydrogenated Soybean and Linseed Esters: Special Lubricants. J. Am. Oil Chem. Soc. 53 (1976):511-517.

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SCHWAB, A. W., L. E. GAST, AND W. K. ROHWEDDER. Nucleophilic and Radical Addition of H_2S to Methyl Linolenate and Linseed Oil. J. Am. Oil Chem. Soc. 53 (1976):762-766.

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GAST, L. E. Potential Industrial Products from Linseed Oil. Presented at the Flax Institute of the United States. Minot, North Dakota, November 1112, 1976.

SCHNEIDER, W. J. AND L. E. GAST. Water-Dispersible Urethane-Polyesteramide Coatings from Linseed Oil. Presented at the Meeting of the American Oil Chemists' Society, Chicago, Illinois, September 1976.

20540-013

Publications:

NELSON, G. E. N. AND R. E. PETERSON. L-Serine Dehydratase from Proteus vulgaris. Dev. Ind. Microbiol. 17 (1976):399-404.

NELSON, G. E. N. AND R. E. PETERSON. L-Serine Dehydratase Production by Aeromonas punctata. Dev. Ind. Microbiol. In press.

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NELSON, G. E. N. AND R. E. PETERSON. L-Serine Dehydratase Production by Aeromonas punctata. Presented at the Society for Industrial Microbiology Meeting, Jekyll Island, Georgia, August 15-20, 1976.

20540-014

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MIWA, T. K. AND J. W. HAGEMANN. Physical and Chemical Properties of Jojoba Liquid and Solid Waxes. Proceedings of the Second International Conference on Jojoba and Its Uses, February 10-12, 1976, Ensenada, Mexico. Accepted December 29, 1976.

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SIMPSON, T. D. AND T. K. MIWA. X-ray Study of Hydrogenated Jojoba Wax. J. Am. Oil Chem. Soc. 54 (1977):54-58.

20540-016

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PRYDE, E. H., L. E. GAST, E. N. FRANKEL, AND K. D. CARLSON. Unsaturated Vegetable Oils as Renewable Resources for Plastics Applications. Polym. Plast. Technol. Eng. 7 (1976):1-26.

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FRANKEL, E. N. AND E. H. PRYDE. Catalytic Hydroformylation and Hydrocarboxylation of Unsaturated Fatty Compounds. Presented at the symposium on Industrial Uses of Fats and Oils

at the American Oil Chemists' Society, New Orleans, Louisiana, April 21-25, 1976.

KOHLHASE, W. L., E. N. FRANKEL, AND E. H. PRYDE. Polyamides for Carboxystearic Acid. Presented at the meeting of the American Oil Chemists' Society, Chicago, Illinois, September 26-29, 1976.

MILLER, W. R. AND E. H. PRYDE. Geminal Hydroxymethyl Compounds from 9(10) Formylstearic Acid. Presented at the symposium on Industrial Uses of Fats and Oils, American Oil Chemists' Society, New Orleans, Louisiana, April 21-24, 1976.

PRYDE, E. H. Current and Future Research on Fats and Oils. Presented at a meeting of the Fatty Acid Producers Council, SRRC, New Orleans, Louisiana, April 21, 1976.

PRYDE, E. H. Nonfood Uses for Commercial Vegetable Oil Crops. Presented at the Symposium on "C.R.O.P.S. - Crop Resources: Origin and Potential to Society" at the Annual Meeting for the Society for Economic Botany, Urbana, Illinois, June 13-16, 1976.

20540-017

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RANKIN, J. C., B. S. PHILLIPS, W. M. DOANE, AND C. R. RUSSELL. Steam Jet Cooking of Unmodified and Modified Cereal Flours: Evaluation of Paste Properties, Papermaking Uses, and Flocculation Activity. Staerke 28 (1976):174-179.

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Publications:

WING, R. E. Corn Starch Compound Recovers Metals from Water. Ind. Wastes 21 (1975):26-27.

WING, R. E. AND W. M. DOANE. Removal of Heavy Metal Ions from Aqueous Solutions with Insoluble Crosslinked Starch Xanthate. U.S. Patent 3,979,286, September 7, 1976.

WING, R. E. Starch Purifies PC Rinse Waters. Circuits Manuf. 16 (1976):10, 12, 14-16.

WING, R. E., W. E. RAYFORD, AND W. M. DOANE. Treatment Process for Copper Pyrophosphate Electroplating Rinse Waters. Met. Finish. In press.

WING, R. E., W. E. RAYFORD, AND W. M. DOANE. Treatment Process for Some Rinse Waters from the Electroless Plating of Copper. Plat. Surf. Finish. In press.

WING, R. E., W. E. RAYFORD, W. M. DOANE, AND C. R. RUSSELL. Preparation of Insoluble Cationic Starches and Their Use in Heavy Metal Anion Removal. J. Appl. Polym. Sci. In press.

WING, R. E. Starch-Based Products in Heavy Metal Removal. Chapter in "Ion Exchange Processes in Pollution Control," C. Calmon and H. Gold (Eds.), Chemical Rubber Co. pubs. In press.

Other Reports:

WING, R. E. AND W. E. RAYFORD. Heavy Metal Removal from Printed Circuit Rinse Waters. Presented at Fall Institute of Printed Circuits Meeting, San Francisco, California, September 27-30, 1976.

WING, R. E. AND W. E. RAYFORD. Heavy Metal Pollution Control in the Printed Circuit Industry. Presented at 6th Annual Merrimack Valley Branch, American Electroplaters Society Workshop, Danvers, Massachusetts, March 9-10, 1977.

20540-020

Publications:

DENNENBERG, R. J., R. J. BOTHAST, AND T. P. ABBOTT. A New Biodegradable Plastic Made from Starch Graft Poly(methyl acrylate) Copolymer. J. Appl. Polym. Sci. In press.

OTEY, F. H. Current and Potential Uses of Starch Products in Plastics. Polym.-Plast. Technol. Eng. 7 (1976):221-234.

OTEY, F. H. AND A. M. MARK. Degradable Starch-Based Agricultural Mulch Film. U.S. Patent 3,949,145, April 6, 1976.

OTEY, F. H., C. A. WILHAM, A. M. MARK, AND C. R. RUSSELL. Halogenated Polyallyl Ethers in Flame-Retardant Urethane Foams. Ind. Eng. Chem., Prod. Res. Dev. 15 (1976):183-186.

Other Reports:

OTEY, F. H. Cereal-Based Films and Plastics. Presented at the 17th Annual Corn Dry Milling Conference, Peoria, Illinois, June 2-3, 1976.

OTEY, F. H. New Industrial Potentials for Carbohydrates. Invited presentation, Symposium on "C.R.O.P.S. (Cultivated Resources: Origin and Potential for Society)," Annual Meeting of the Society of Economic Botany, Urbana, Illinois, June 13-16, 1976.

Considerable time and effort were devoted to general public and industrial inquiries relating to the subject of starch-based plastics. One company reports that they have made 20,000-lb batches of starch-derived polyethers, by our patented process, which were shipped to foam customers for evaluation. The Vice-President of Mono-Sol wrote a letter expressing appreciation for our contribution to the development of starch-polyvinyl alcohol films. The company is using an estimated 1 million pounds of starch per year to make water-soluble bags and expects to increase starch consumption in this area during the next year. Using starch in these bags not only reduces pollution hazards because they are biodegradable but also conserves 1 million pounds of petroleum products formerly used to make the bags.

3. Plan of Work:

20540-002

None under A_2. See A_1 3 for specific objectives and plans of work.

20540-003

a. Specific Objective: Examine the graft polymerization of butadiene onto starch.

   Plan of Work: Butadiene will be graft polymerized onto starch via cobalt-60 initiation, both by itself and in the presence of active monomers such as acrylonitrile of styrene. The extrusion processing of these graft copolymers into useful plastics and/or rubbers will be examined.

b. Specific Objective: Examine the graft polymerization of vinyl acetate onto starch.

   Plan of Work: Vinyl acetate will be graft polymerized onto starch via cobalt-60 initiation, both by itself and in the presence of active monomers such as acrylamide. Products will then be treated with alkali to yield water-dispersible starch-poly(vinyl alcohol) graft copolymers. Use of these graft copolymers as coating adhesives, films, and yarn sizings will be examined.

c. Specific Objective: Promote the commercialization and use of saponified starch-polyacrylonitrile (Super Slurper).

Plan of Work: Correspondence relating to Super Slurper will be answered with reprints of our published work and samples of the absorbent polymer. We will cooperate with other agencies to study the uses of Super Slurper in agriculture and will run additional reactions in our pilot plant when necessary to supply pound quantities of the absorbent polymer for cooperative studies. We will continue to seek ways to improve upon and make more economical the synthesis of Super Slurper.

20540-008

Specific Objective: Explore feasibility for using crude enzymes to cut processing cost.

Plan of Work: A simple fluidized bed reactor will be used to evaluate the efficacy of an enzyme-resin complex made with crude enzyme preparations. Higher throughput rates may obviate the deterioration problems now encountered with commercial white water. Evaluation of effectiveness of treatment will be based on alum requirement for clarification to a designated extent. If a viable system is evolved, the enzyme-resin half-life will be estimated.

20540-009

a. Specific Objective: Establish material balances for the modified (closed loop) papermaking system.

Plan of Work: Pulp, water, and chemicals to the machine will be balanced against paper produced and effluents discharged to the sewer.

b. Specific Objective: Determine the time required to achieve static conditions within the white-water recycling loop for various grades of paper and machine operating conditions.

Plan of Work: Fiber build-up and chemical content will be monitored at the wire, silo, broke pit, seal pit, and secondary cleaners during prolonged machine runs to establish the time required to reach conditions of equilibrium within the white-water loop.

c. Specific Objective: Determine the effects of the closed white-water system on machine operation and paper quality in prolonged runs.

Plan of Work: After the time to reach equilibrium has been reached (item b), machine runs that exceed this time will be made to determine effects of the closed system on physical properties of paper produced and operational characteristics of the machine. These studies may be repeated for several grades of paper, ranging from coarse to highly filled fine papers.

20540-017

a. Specific Objective: Evaluate quaternary cationic flours and starches, before and after enzyme degradation as sizes in wet- and dry-end applications on the NRRC pilot paper machine.

Plan of Work: The quaternary cationic products will be evaluated in wet-end and surface sizing applications using the NRRC pilot paper machine to determine their effectiveness of pollution abatement (reduction of losses in wastewater), improvement of paper properties, and potential for upgrading recycled paper.

b. Specific Objective: Prepare starch derivatives with pendant epoxide and N-methylol groups for evaluation as permanent sizing agents for textiles. In addition, prepare and test other cationic, anionic, and amphoteric starch derivatives designed as warp sizes for textile yarn.

Plan of Work: The bifunctional agents glycidyl acrylate and N-methylol acrylamide will be reacted with starch to provide products with sites that are capable of reacting with the hydroxyl groups of the cellulose fibers. Products will be padded onto the yarn and heat cured to crosslink with the fibers. Ionic derivatives of starch, prepared by dry and wet methods, for warp sizing of textile yarns will be evaluated as sizing agents and those that show promise will be further tested as to their recoverability and reuse. Samples of promising candidates will be provided to the ARS Southern Regional Research Center (SRRC) for evaluation as textile sizing agents.

c. Specific Objective: Prepare cereal-based cationic, anionic, and amphoteric graft copolymers by dry-state reaction conditions and evaluate selected products as paper and textile sizes.

Plan of Work: Dry-state preparation of cationic, anionic, and amphoteric cereal-based graft copolymers will be carried out by reacting flours and starches with selected acrylic, vinylic, and allylic monomers employing alkali, Lewis acids, and redox catalyst systems. The cereal will be dry blended with catalyst and various amounts of monomer reagent and reacted under various conditions of time, temperature, and pH. For example, 2-hydroxy-3-methacryloxypropyltrimethylammonium chloride and 2-acrylamido-2-methyl propane sulfonic acid, reacted with the cereal under the above range of dry-state conditions, have shown promise. Graft polymer products will be evaluated relative to other experimental and commercial paper and textile sizes to provide comparative cost-performance data. Cooperation with SRRC will be maintained to optimize performance of selected products, especially with respect to potential textile sizes.

20540-018

None under A_2. See A_1 3.

20540-019

a. Specific Objective: Prepare and evaluate water-soluble and water-insoluble starch based polymers as flocculating agents and heavy metal removal agents.

Plan of Work: Starch-based products having higher molecular weights will be prepared and screened as flocculating agents for celite, silica, and heavy metal hydroxide systems. Starch-based products containing such groups as xanthide, carboxyl, amine, and ammonium will be prepared by standard techniques and evaluated for efficacy in removing heavy metals from industrial wastewaters.

b. Specific Objective: Promote promising new products to industry to enhance commercial acceptance.

Plan of Work: Contacts will be established with appropriate industries, and information and samples will be provided as requested. Visits will be made to selected industrial sites to encourage on-site industrial tests of our products.

20540-020

a. Specific Objective: Complete the evaluation of halogenated products, made from methyl glucoside polyethers, as flame-retardants in urethane foams.

Plan of Work: Incorporate these new halogenated products into urethane foams and determine the humid age stability, strength, and flame-resistance of the foams and prepare a manuscript on the results.

b. Specific Objective: Continue evaluation of starch films made with ethylene acrylic acid copolymers for mulching and packaging applications.

Plan of Work: Expose the starch films to outdoor weathering and soil microorganisms and correlate the rate of degradation and physical properties to starch content.

c. Specific Objective: Develop improved plasticizer systems for starch-polyvinyl alcohol (PVA) films for packaging applications that will extend the shelf life of starch films.

Plan of Work: Prepare a variety of starch-PVA films using several different polyol combinations as plasticizers and correlate the effects of these polyols on the strength and percentage elongation of films during extended aging.

B. Summary of Progress for Extramural Projects:

1. Progress Report (Narrative):

3090-20541-021-C

No commercial-scale crushing of crambe seed was carried out in 1976 under the Agricom contract although samples analyses were completed for the run originally made in December 1975 at Culbertson, Montana.

Moisture, oil, free glucose, and glucosinolate were some of the analyses completed on 49 crambe meal samples collected during the December 1975 crush in Culbertson, Montana. The samples included two wholeseed samples, 22 expeller meals, 8 extractor meals, and 17 finished (D-T) meals. Average seed entering the plant contained only 67-73% crambe seed, the remainder being chaff and about 10 types of foreign seeds. The average oil and glucosinolate levels in the four types of meals sampled were: whole seed, 27.4 and 5.4%; expeller meals, 19.5 and 5.0%; extractor meals, 3.3 and 4.7%; and D-T meals, 3.7 and 1.3%, respectively. Glucosinolate, nitrogen extractability, and lysine levels did not change appreciably during the expelling and extracting phases, but all three decreased significantly in the desolventizing and toasting phase. Since the moisture and quality of the seed varied considerably, only intermittent success was realized in inactivating

the glucosinolase enzyme system. Erucic acid content of the oil (51%) and protein content of the meal (27%) were lower than anticipated because of inadequate seed cleaning prior to the expeller operations. The significant amount of flax seed in the plant feed raised the linolenic acid content of the oil to 9% and the iodine value to 96-99. Sufficient seed was not harvested in 1976 to warrant a crush, but one is anticipated in 1977 or early 1978.

8003-20540-041

The previously developed selective hydrogenation procedure was successfully extended to preparation of α-dihydropolyprenols from plant prenols. A C-55 dihydroundecaprenol phosphate of this type served as liquid precursor of lipid-linked oligosaccharides in yeast. It also substituted for the natural C-95 analog in synthesis of lipid-linked oligosaccharides in mammalian systems. Sugar transferases dependent on the presence of polyprenol phosphate were found in yeast nuclear and mitochondrial fractions. Alkaline enolization was studied as a means of chemically isomerizing single isoprene residues of polyprenols.

8002-20540-240

Samples of dung, soil, food grains, and decaying vegetables and fruits were collected from nine districts in Pakistan. From these collections, about 150 isolates of Mucorales were obtained and over 100 of these have been identified. They belong to 51 species. Thirty-four belong in the family Mucoraceae, eight in Pilobolaceae, two in the Thamnideaceae, three in Cunninghamellaceae, one in the Synecephalastraceae, and three in Piptocephalidaceae. Of these 51 species, 18 had never been encountered in Pakistan before. Several of these may represent new species.

2. Progress Reports (Achievements):

8003-20540-041

Publications:

CHOJNACKI, T., W. JANKOWSKI, T. MANKOWSKI, AND W. SASAK. Preparative Separation of Naturally Occurring Mixtures of Polyprenols on Hydroxyalkoxypropyl-Sephadex. Anal. Biochem. 69 (1975):114-119.

SASAK, W., T. MANKOWSKI, AND T. CHOJNACKI. Polyprenols in Juniperus communis Needles. FEBS Lett. 64 (1976):55-58.

MANKOWSKI, T., W. JANKOWSKI, T. CHOJNACKI, AND P. FRANKE. C-55 Dolichol: Occurrence in Pig Liver and Preparation by Hydrogenation of Plant Undecaprenol. Biochemistry 15 (1976):2125-2130.

TECHNOLOGIES FOR FIBER USES

A_1 Technological Objective: Develop improved methods for separating and cleaning agricultural fibers and for preparing them for conversion in yarns, webs, and fabrics.

1. Progress Report (Narrative):

20550-001

a. Specific Objective: Produce pulp for use in formulation of furnishes containing large proportions of kenaf fiber.

Progress: One-half ton of thermal mechanical pulp was produced in our industrial cooperator's digester. With the same equipment, our endeavors to prepare bleachable and bag grade chemical pulps were frustrated by severe plugging caused by the engineering design of the pilot-plant equipment. The bridging and subsequent plugging was aggravated by the facility with which kenaf hydrates and softens.

b. Specific Objective: Prepare bleached pulp.

Progress: In the laboratory, soda pulp, 19.2 kappa number, was bleached with single stage hypochlorite (H) to 71-74% brightness in 94-98% yield. A two-stage H-H gave 93% yield with 81% brightness. A three-stage H-extraction-H gave 93% yield with 82% brightness. Chemical consumptions were 6.0, 6.5, and 6.5%, respectively. Bleaching of 1 ton of bleachable grade of pulp is contingent upon its production in commercial equipment (see a).

c. Specific Objective: Prepare a web containing bleachable kenaf pulp for demonstration of its suitability in commercial printing trials.

Progress: The bleachable grade of kenaf pulp in a was not produced; however, work has progressed toward the preparation of newsprint web using thermal mechanical pulp. Commercial newsprint arranged for by memorandum and experimental thermal mechanical newsprint produced from loblolly pine by Finnish industry were tested to provide internal comparison data. The NRRC pilot paper machine was modified to provide a 15-inch trimmed web. Repulped newsprint was run to gain operator experience with typical furnishes.

d. Specific Objective: Evaluate a web containing 40 to 50% bleached kenaf pulp on a commercial printing press.

Progress: The bleachable grade of kenaf pulp in a was not produced; however, memoranda have been signed to allow evaluation of newsprint furnishes containing kenaf thermal mechanical pulp.

e. Specific Objective: Continue studies to determine consequences on composition, physical, and mechanical properties, and pulping characteristics of growing kenaf on reclaimed strip-mine land.

Progress: Kenaf grown in Illinois on four conditions of fertilization was harvested 1 month after a killing frost. Yields were 4500, 4100, 3800, and 2700 pounds/acre, respectively, from plots having treatment variables of two levels of activated sludge, commercial fertilizer, and no fertilizer. Soil samples were collected for analyses.

f. Specific Objective: Coordinate continuing studies on kenaf with cooperating plant geneticists, agronomists, and agricultural engineers.

Progress: Kenaf (variety Everglades 71) grown in Indiana, Maryland, and Georgia had bark thickness proportional to stalk diameter (11 to 35 mm).

Since the density and bulk of kenaf is about one-third those of typical papermaking wood species, compaction was studied. Bulk density of kenaf was increased sixfold by high-density baling. Cubes formed in commercial equipment had a bulk density 3-1/2 times that of chopped kenaf.

Samples have been removed from stacks (15 to 18 tons) established to evaluate the influence of stack size on preservation. Included as variables in these tests were moisture (75 and 25%) and chemical preservatives (borax and sodium bisulfite).

g. Specific Objective: Study contribution of kenaf bark and core fiber to papermaking systems.

Progress: Pulps were prepared under identical conditions from kenaf and its bark and core and the pulps were formed into webs. Scanning electron microscopy revealed that core pulp collapse was more complete during sheet formation. That observation was further substantiated by

density measurements. Calendering increased densities as follows: kenaf core, 6.8%; kenaf bark, 10.9%; and whole kenaf, 14.4%. Laboratory data for the correlation of cooking conditions and pulp properties have been obtained for kenaf core at 155°, 162.5°, 170°, 177.5°, and 185° C. This information will be used to determine pulping kinetics and to guide commercial scale batch and continuous cooking mode.

2. Progress Reports (Achievements):

20550-001

Publications:

BAGBY, M. O. AND T. F. CLARK. Kenaf for Hardboards. TAPPI CA Report No. 67, Nonwood Plant Fiber Pulping--Progress Report No. 7 (1976):9-13.

Other Reports:

TOUZINSKY, G. F., F. L. BAKER, R. L. CUNNINGHAM, AND M. O. BAGBY. Scanning Electron Microscopy of Kenaf Paper Structures. Abstract of Papers, 172nd ACS National Meeting, Cellulose, Paper, and Textile Division, San Francisco, California, August 29-September 3, 1976. Abstract 59.

3. Plan of Work:

20550-001

a. Specific Objective: Clean thermal mechanical pulp prepared by industrial equipment.

Plan of Work: On a pilot scale, design a system to clean and extract thermal mechanical pulp with minimum loss of fiber and process 1/2 ton of pulp.

b. Specific Objective: Bleach thermal mechanical pulp.

Plan of Work: Bleach about 1/2 ton of thermal mechanical pulp with chemicals common to the newsprint industry. Establish the preferred bleaching sequence on a laboratory scale.

c. Specific Objective: Prepare newsprint web from kenaf pulps, thermal mechanical and chemical.

Plan of Work: Produce commercial size rolls of kenaf newsprint containing bleached thermal mechanical pulp and bleached soda pulp. Establish the furnish formulations and refining treatment on a laboratory scale. Produce commercial sized comparison roll of newsprint from recycled commercial newsprint.

d. Specific Objective: Evaluate a newsprint web containing kenaf thermal mechanical pulp on commercial printing presses.

Plan of Work: Run commercial sized rolls of paper on commercial printing presses and contrast performances of kenaf web with web produced by NRRC from repulped newsprint and commercial newsprint. Plan for additional tests with new furnishes.

e. Specific Objective: Produce pulp for use in formulation of furnishes containing large portions of bleachable grade of kenaf fiber.

Plan of Work: On a pilot-plant scale (by hire), process kenaf by soda technique to give 1/2 to 2 tons of bleached pulp.

f. Specific Objective: Continue experiments with kenaf on strip-mine land to broaden data base.

Plan of Work: The same plots used in 1976 will be planted to kenaf employing identical fertilizer, herbicide, and sludge treatments. Plant yields will be determined. Soil samples and plant materials will be collected for heavy metal analyses, and those samples collected in 1976 will be analyzed.

g. Specific Objective: Coordinate continuing studies on kenaf with cooperating plant geneticists, agronomists, and agricultural engineers.

Plan of Work: Monitor effects of (1) genetic modifications to achieve nematode resistance, (2) agronomic parameters, (3) harvesting methods, and (4) storage conditions on chemical composition, physical and mechanical properties, and pulping.

h. Specific Objective: Complete study on contributions of kenaf bark and core fibers to papermaking systems.

Plan of Work: With major emphasis on parts a-g, determine response to mechanical beating of kenaf bark and core fibers. Determine appropriate cooking conditions and cook at constant kappa numbers. Refine pulps separately and in admixtures. Study their papermaking properties on a fourdrinier paper machine by scanning electron microscopy and by physical measurements.

TECHNOLOGIES FOR MARKETING OF FIELD CROPS

A. Technological Objective: Develop new and improved technologies for maintaining product quality and reducing losses in the marketing channels.

1. Progress Report (Narrative):

20590-001

a. Specific Objective: Control microbial growth in high-moisture corn so that low-temperature drying of the corn, and consequent conservation of high-grade fossil fuels and corn quality become a practical process.

Progress: Intermittent application of gaseous ammonia (0.009-0.09% db) to 560 bushels of high-moisture corn permitted low flow (1.8 M^3/min/1000 kg) ambient drying. Moisture content was reduced from 23.3 percent to 17.7 percent in 56 days and mold growth was effectively controlled throughout 6 months' storage. Total drying costs were 3.6¢/bushel. Feedlot steers gained fast (2.86 pounds/day) and efficiently (6.57 pounds feed/pound gain) on corn dried with this "trickle-ammonia process."

Methylene-bis-propionate (MBP) exhibited superior antifungal and antibacterial properties to gaseous ammonia and liquid anhydrous ammonia in field test for preserving high-moisture corn. However, feedlot performance of steers fed rations formulated with ammonia-treated corn was slightly better than the performance of steers fed MBP-treated corn.

Ammonia application at a rate of 0.05 percent/day (wt NH_3/wt corn) was necessary to eliminate all molds on 25-percent moisture corn during a 10-day laboratory test under ideal conditions for mold growth. However, an application rate of 0.005 percent/day effectively eliminated certain species of Penicillium and Nigrospora. In contrast, species of Fusarium and Cephalosporium survived. Similar experiments with sulfur dioxide indicated that 0.6 percent (wt SO_2/wt corn), applied over a 24-hour period, reduced mold counts from 10^5/g to a nondetectable level and bacterial counts from 10^6 to 10^2/g. Mixtures of SO_2 and NH_3 were less effective than either NH_3 or SO_2 alone for controlling growth of microorganisms on high-moisture corn. Liquid application of formaldehyde, followed by gaseous application of NH_3 to high-moisture corn, increased control of bacteria over NH_3 alone.

b. Specific Objective: Investigate solid substrate fermentation of ammonia-treated grain with Scopulariopsis brevicaulis to improve the nutritional quality of the grain and minimize costs.

Progress: Scopulariopsis brevicaulis was grown on ammonia-treated corn in 2800 ml Fernbach flasks to provide 25 kg of fermented corn for feeding trials. S. brevicaulis has also been grown successfully on ammonia-treated corn in a tank reactor fitted with a vertical central auger. The 25 kg reactor is aerated, temperature-controlled, and the auger agitation is controlled with a timer.

c. Specific Objective: Examine the fungal flora of soybeans and study the effects of spoilage fungi on soy-flavor compounds in order to reduce storage losses.

Progress: A test shipment of soybeans was sampled during loading at Toledo, Ohio, and during unloading at Rotterdam, The Netherlands, to determine the effect of shipment on the microflora of soybeans. Little or no change occurred in the bacterial, mold, and actinomycete content of whole soybeans. However, damaged beans had higher bacterial counts upon arrival in Holland. Common fungi observed were species in the Aspergillus glaucus group and species of Penicillium.

The total carbonyl and the monocarbonyl content of soybeans increased during 10 controlled fungal fermentations. Aspergillus candidus, Penicillium meleagrinum, Rhizopus oligosporus, and Aspergillus flavus increased monocarbonyls at least threefold. However, the effect of fungi on methyl ketones, saturated aldehydes, 2-enals, 2,4-dienals, and the relative concentration of compounds within a separated class, varied from one species to another.

2. Progress Reports (Achievements):

20590-001

Publications:

BOTHAST, R. J., L. T. BLACK, L. L. WILSON, AND E. E. HATFIELD. Methylene-bis-propionate (MBP) Preservation of High Moisture Corn. J. Anim. Sci., in press.

BOTHAST, R. J., M. L. GOULDEN, O. L. SHOTWELL, AND C. W. HESSELTINE. Aspergillus flavus and Aflatoxin Production in Acid-Treated Maize. J. Stored Prod. Res. 12 (1976):177-183.

BOTHAST, R. J. AND K. L. SMILEY. Metabolites of Fungi Used in Food Processing. In Food and Beverage Mycology, Chapter 13. The Avi Publishing Company, Inc., Westport, Connecticut, in press.

DENNENBERG, R. J., R. J. BOTHAST, AND T. P. ABBOTT. A New Biodegradable Plastic Made from Starch Graft Poly(methyl acrylate) Copolymer. J. Appl. Polym. Sci., submitted.

HESSELTINE, C. W. AND R. J. BOTHAST. Mold Development in Ears of Corn from Tasseling to Harvest. Mycologia 69 (1977):in press.

HESSELTINE, C. W., O. L. SHOTWELL, W. F. KWOLEK, E. B. LILLEHOJ, W. K. JACKSON, AND R. J. BOTHAST. Aflatoxin Occurrence in 1973 Corn at Harvest. II. Mycological Studies. Mycologia 68 (1976):341-353.

LANCASTER, E. B., AND R. J. BOTHAST. Treating Maize with Ammonia--A Controlled Storage Experiment. J. Stored Prod. Res. 12 (1976):171-175.

NOFSINGER, G. W., R. J. BOTHAST, E. B. LANCASTER, AND E. B. BAGLEY. Ammonia-Supplemented Ambient Temperature Drying of High-Moisture Corn. Trans. ASAE, submitted.

PEPLINSKI, A. J., O. L. BREKKE, R. J. BOTHAST, AND L. T. BLACK. High Moisture Corn--An Extended Preservation with Ammonia. Trans. ASAE, submitted.

Other Reports:

BOTHAST, R. J. Methylene-bis-Propionate Preservation of High-Moisture Corn. Presented at American Society of Animal Science Meeting, College Station, Texas, August 15-18, 1976.

HESSELTINE, C. W. Mold Development in Growing Corn. Presented at American Society for Microbiology Meeting, Atlantic City, New Jersey, May 2-7, 1976.

NOFSINGER, G. W. Ammonia-Supplemented Ambient Temperature Drying of High-Moisture Corn. Presented at American Society of Agricultural Engineers Meeting, Chicago, Illinois, December 14-17, 1976.

3. Plan of Work:

20590-001

a. Specific Objective: Develop NRRC's "trickle-ammonia process" for ambient-air drying of high-moisture corn by including other microbial inhibitors in large-scale tests and seek Environmental Protection Agency approval for the process.

Plan of Work: Intermittent gaseous application of ammonia and sulfur dioxide will be examined independently and in conjunction with initial applications of formaldehyde and methylene-bis-propionate during ambient-air drying of high-moisture corn in 1500bushel tests. Airflow rates from 2-4 cfm/bushel will be evaluated. Feedlot performance will be determined on steers fed rations formulated with corn from these treatments.

b. Specific Objective: Investigate solid substrate fermentation as a process for improving the protein content of grain and producing metabolites.

Plan of Work: The effect of inoculum, agitation, aeration, moisture, temperature, ammonia, and reactor configuration will be investigated. Products will be determined analytically. Ammonia-treated corn, fermented with Scopulariopsis brevicaulis, will be evaluated through chicken-feeding tests.

c. Specific Objective: Solve emergency problems associated with microbial deterioration and contamination in food and grain.

Plan of Work: Two types of polyethylene-coated, multiwall paper containers and a conventional container will be compared for resistance to fungal penetration during an international test shipment of corn soya milk. The mode of Aspergillus flavus invasion into the corn kernel will be investigated.

RESEARCH TO IMPROVE HUMAN HEALTH AND SAFETY

CRIS Work Units Listed by Work Reporting Unit

Work Reporting Unit (WRU)

Number	Title (Principal Investigator)
CHEMICAL RESIDUES AND ADDITIVES IN FOOD AND FEED (p. 132)	
3102-20820-001	Environmental contaminants in cereals and processed cereal foods (C. W. Blessin)
NATURAL TOXICANTS AND MICROBIAL TOXINS (p. 134)	
3102-20840-001-A	Cattle feeding tests of crambe meal as a protein concentrate (G. C. Mustakas and L. H. Princen)
3102-20840-002	Hazardous products of interactions between natural toxins and cereal grain components (A. Ciegler)
3102-20840-003	Fungal production of toxic macromolecules (R. W. Detroy)
3102-20840-004	Glucosinolates and derived substances in cruciferous foods and feeds (C. H. VanEtten)
3102-20840-005	Studies on mycotoxins in cereal grains and their control (C. W. Hesseltine and A. Ciegler)
3102-20840-006	Investigation of potential mycotoxin formation in wild rice processing (A. Ciegler)
8004-20840-008	Establish and identify toxic metabolites of certain molds and aflatoxin in livestock fodder (C. W. Hesseltine)

CHEMICAL RESIDUES AND ADDITIVES IN FOOD AND FEED

A. Technological Objective: Devise means to reduce or eliminate hazardous environmental contaminants in food and feed products.

1. Progress Report (Narrative):

20820-001

Specific Objective: Continue research on changes in composition of trace elements, including heavy metals, in cereals and other crops grown on normal soil types with mineral and organic fertilizers.

Progress: In studies with corn, excessive quantities of seven heavy metals (Zn, Mn, Cu, Pb, Cr, Cd, and Hg) were excluded from the kernels by plants grown on untreated and sewage sludge amended strip-mine soil.

Thus, no hazard to animals or humans could be ascribed when these grains enter the food chain. In addition, the cob, husk, upper and lower stems, while generally having higher metal concentrations than the kernel show no excessive accumulation of metals and could be fed to animals. However, in the case of leaves, and especially roots, translocation increased as a direct result of the sludge application, but no evidence of phytotoxicity was observed.

The root should probably be eliminated as an animal feed component, since concentrations of copper and cadmium could become excessive in this tissue, where the added sludge contains high concentrations of these two elements, and where the availability of the metal in the amended soil matrix was demonstrated to be high.

The element of greatest concern to the food chain is cadmium. Shown most dramatically in this study is the increased availability of cadmium to the corn plant and its subsequent incorporation into specific plant tissues. Cadmium could pose a special problem to animal feeding where the total corn plant is used; however, consumption of the grain by animals and humans as grown under the conditions described here would constitute no hazard.

2. Progress Report (Achievements):

20820-001

Publications:

None

Other Reports:

GARCIA, W. J., C. W. BLESSIN, AND G. E. INGLETT. Distribution of Zinc-65 as Incorporated into the Germ, Endosperm-Hull and Oil Fractions of Corn. Presented at the Annual Meeting, American Association of Cereal Chemists, New Orleans, Louisiana, October 5-8, 1976.

BLESSIN, C. W., W. L. DEATHERAGE, AND G. E. INGLETT. Preparation and Properties of Defatted Flours from Dry-Milled Yellow, White and High-Lysine Corn Germ. Presented at the Annual Meeting, American Association of Cereal Chemists, New Orleans, Louisiana, October 5-8, 1976.

BLESSIN, C. W. Corn Germ Flour. Presented on local portion of television program "Today Show," WEEK, Channel 25, Peoria, Illinois, June 17, 1976.

3. Plan of Work:

20820-001

Specific Objective: Conduct research on relative abundance of heavy metals in selected food crops grown on normal soils with mineral and organic fertilizers to identify crops with unfavorable cadmium to zinc ratios.

Plan of Work: Determine concentration of trace elements including heavy metals in selected above ground and root crops grown on black, brown, and clay soil types with mineral fertilizers and digested sewage sludge.

The following crops: beans, beets, cabbage, carrots, cauliflower, eggplant, lettuce, onions, peas, peppers, radishes, spinach, sweet corn, tomatoes, and wheat will be examined. Analytical work will include an investigation of Cd to Zn ratios in selected crops.

NATURAL TOXICANTS AND MICROBIAL TOXINS

A_1 Technological Objective: Assess the health hazards of naturally occurring toxicants in food and feed and reduce or eliminate mycotoxins and toxigenic fungi.

1. Progress Report (Narrative):

20840-001A

Specific Objective: Repeat cattle-feeding trials to show the efficacy of crambe meal as a supplement in a protein-deficient diet.

Progress: Since crambe meal from a cooperative crushing program became available so late that the experimental beef animals were too heavy and the proper number of animal pens were no longer available, the feeding trial was postponed until new young animals and the required pens became available. Consequently, the trial was started on October 28, 1976, and is still in progress. Preliminary data appear promising.

20840-004

a. Specific Objective: Determine the effect of storage of cabbage heads on their glucosinolate content and the effect of glucosinolate content on the cabbage keeping quality during storage.

Progress: Based on storage of 10 varieties by two commercial methods as analyzed at 1, 3, and 6 months' storage time, there was evidence for a slight but not significant decrease in total glucosinolate content as well as a change in the relative composition. Total glucosinolates or differences in kinds of glucosinolates were not related to relative keeping qualities of the different varieties. From a practical standpoint, the measured changes are of no consequence and cabbage keeping quality appears to be not related to glucosinolate content.

b. Specific Objective: Continued the survey on glucosinolate content of vegetables from the Cruciferae family.

Progress: Laboratory work on the glucosinolate content was completed for 46 varieties of cabbage, 12 of which were repeats, grown in 1975, of the 22 varieties previously reported, as grown in 1974. Also 18 varieties of Chinese cabbage, 12 of radish, 13 of turnip, 3 of rutabaga, 4 of kale, 3 of collards, and 1 variety of kohlrabi were analyzed. Interpretation of the results requires assembly and statistical evaluation of the data.

c. Specific Objective: Isolate in sufficient quantity for biological testing specific glucosinolates and hydrolysis products.

Progress: The compound 1-cyano-3,4-epithiobutane has been isolated pure in sufficient quantity for biological testing for teratogenic activity by Dr. Keyl's group at the ARS Russell Research Center, Athens, Georgia, and for the Salmonella test for mutagenesis and carcinogenesis by Dr. Gumbman's group at the ARS, Western Regional Research Center. Crystalline (R) goitrin and epi-progoitrin have also been prepared to send to Dr. Keyl's laboratory.

2. Progress Reports (Achievements):

Publications:

20840-001A

VanETTEN, C. H., M. E. DAXENBICHLER, W. SCHROEDER, L. H. PRINCEN, AND T. W. PERRY. Tests for epi-Progoitrin, Derived Nitriles, and Goitrin in Body Tissues from Cattle Fed Crambe Meal. Can. J. Anim. Sci., in press, March (1977).

20840-004

VanETTEN, C. H., M. E. DAXENBICHLER, P. H. WILLIAMS, AND W. F. KWOLEK. Glucosinolates and Derived Products in Cruciferous Vegetables: Analysis of the Edible Part from 22 Varieties of Cabbage. J. Agric. Food Chem. 24 (1976):452.

DAXENBICHLER, M. E., C. H. VanETTEN, AND G. F. SPENCER. Glucosinolates and Derived Products in Cruciferous Vegetables. Identification of Organic Nitriles from Cabbage. J. Agric. Food Chem. 25 (1977):121.

DAXENBICHLER, M. E. AND C. H. VanETTEN. Glucosinolates and Derived Products in Cruciferous Vegetables: Gas Liquid Chromatographic Determination of the Aglucon Derivatives. J. Assoc. Off. Anal. Chem., in press, July (1977).

VanETTEN, C. H. AND DAXENBICHLER, M. E. Glucosinolates and Derived Products in Cruciferae Vegetables: Total Glucosinolates by Retention of an Anion Exchange Resin and Enzymatic Hydrolysis to Measure the Released Glucose. J. Assoc. Off. Anal. Chem., in press, July (1977).

MUSTAKAS, G. C., L. D. KIRK, E. L. GRIFFIN, JR., AND A. N. BOOTH. Crambe Seed Processing: Removal of Glucosinolates by Water Extraction. J. Am. Oil Chem. Soc. 53 (1976):12-16.

3. Plan of Work:

20840-001A

Specific Objective: Continue cattle-feeding trials with crambe meal for FDA approval.

Plan of Work: Feed cattle for up to 200 days a ration containing sufficient crambe meal to increase the dietary protein content to 10.3 percent from a deficient diet of 9.0 percent protein. Determine the weight gain as a function of time and of feed consumed and compare with animals fed a similar diet containing soybean meal.

20840-004

a. Specific Objective: Continue the survey on glucosinolate content of vegetables from the Cruciferae family.

Plan of Work: Complete analysis of plant samples now on hand. Assemble all the data obtained for evaluation and reporting.

b. Specific Objective: Continue isolation and purification of specific hydrolysis products from the aglucons of glucosinolates for biological testing.

Plan of Work: Select those nitriles for isolation which are formed from the major glucosinolates found in horticultural crops that can be most easily isolated. The unsaturated hydroxy nitrile and the epithio nitriles from epi-progoitrin will be isolated in sufficient amount for testing.

c. Specific Objective: Determine the hydrolysis products formed from the glucosinolates in cabbage during fermentation to convert cabbage to sauerkraut.

Plan of Work: Representative samples of the cabbage used for fermentation of the 1976 crop from Oregon, New York, and Wisconsin have been received and analyzed. We will receive frozen samples of material taken at weekly intervals throughout the sauerkraut fermentation and of the final

product. Completed analyses of the samples should give a picture of the development of hydrolysis products throughout the fermentation and indicate composition changes as related to total acidity and salt content.

d. Specific Objective: Determine the effects of certain processing or pre-processing conditions on the nutritional value of seed meals that may contain toxins.

Plan of Work: Perform analyses for glucosinolates and their products on Limnanthes and Crambe meals which have been exposed to various conditions of heat and moisture. Test autolyzates of raw Jojoba meal for release of glucose as a measure of hydrolysis of the toxic glycoside simmondsin.

e. Specific Objective: Determine the effect of selected germ plasm variation and growing conditions on the amount and composition of the volatile oils in carrot roots.

Plan of Work: Measure the quantity of volatile oil and the content of myristicin and other toxic constituents therein for well-defined inbred lines of carrots. The samples will be grown under commercial conditions in California, Texas, Florida, Wisconsin, and Michigan. Attempt to relate volatile oil content and composition to genetic variation of carrots grown in a single location (Michigan).

A_2 Technological Objective: Identify, control, or eliminate mycotoxins and toxigenic fungi from food and feed.

1. Progress Report (Narrative):

20840-002

Specific Objective: Characterize reaction products between patulin and cysteine.

Progress: Reaction of patulin with cysteine produced up to 11 products detectable by TLC; all adducts were unstable. An acidic adduct isolated by HPLC did not give NMR evidence for a fused ring cyclic lactam with a free carboxyl group but rather a dicarboxylic methyl ester with a free-NH_2. The hemiacetal group of patulin was converted to a lactone and reacted with cysteine. A stable adduct was formed to be used in further studies. Model reactions of patulin with various thiols resulted in products too unstable to isolate.

20840-003

a. Specific Objective: Determine factors involved in fungal virus transmission.

Progress: Transmission experiments were continued with viruses from Penicillium stoloniferum with little success into recipient Penicillium species and yeasts using heterokaryosis and sexual mating techniques. Compatibility factors appear to be involved with virus transfer between eucaryotic organisms such as fungi and yeasts. Attempts were also made with fungal protoplasts via enzymatic cell wall digestion to infect with fungal viruses.

b. Specific Objective: Determine occurrence of mycoviruses in fungal plant pathogens.

Progress: Limited studies with fungal pathogens, although extraction and isolation techniques were developed to begin virus screen with new electron microscope facility.

c. Specific Objective: Determine mycovirus production and maintenance in carrier fungi.

Progress: Micromanipulation techniques were employed to isolate a large number of single-conidia isolates to study virus transmission during conidiation. Random selection of conidia showed that 7 percent of the population no longer harbored virus. Conidiation appears to be most important in the continued persistence of fungal viruses and their ability to replicate.

d. Specific Objective: Determine mycovirus-mycotoxin interactions.

Progress: Various mycotoxins and antimetabolites were evaluated as inhibitors of fungal virus replication. Various intercalating dyes, such as ethidium bromide and acridine orange, were most effective binders to viral dsRNA and effectors of inhibition of viral RNA synthesis. Binding studies with patulin indicated that this mycotoxin is causing viral inhibition other than preferential binding to virus particles alone.

20840-005

a. Specific Objective: Identify hybrid corn lines exhibiting resistance of developing kernels to Aspergillus flavus infection.

Progress: Ten single cross hybrids and six parental inbreds were grown in Georgia, South Carolina, Texas, and Missouri. Ears were inoculated with A. flavus spores 35 days after silking. Test ears were harvested at maturity and sent to NRRC. Corn from these samples is currently being processed and assayed for aflatoxin.

Thirteen single cross hybrids containing different levels of anthocyanins in the kernel aleurone were grown in South Carolina and Florida. Ears were allowed to develop to maturity without treatment. Although seed from three hybrids grown in South Carolina and a single variety in Georgia was not contaminated with aflatoxin, identical hybrids at the alternative growing site did contain the toxin. However, corn from specific hybrids grown at both locations exhibited significantly less toxin than seed from other varieties. Precursors of anthocyanin pigments in certain hybrids may provide developing kernels with some protection from insect and fungal attack. Four commercially grown varieties of white corn were grown at 11 locations in Tennessee, Kansas, and Missouri. The test was designed to provide information on the natural field occurrence of aflatoxin contamination in areas of white corn production. Preliminary results show a broad distribution of the toxin between varieties. Since the assays have not been completed, interregional variation cannot be determined.

b. Specific Objective: Determine environmental effects of date of planting on insect damage and associated A. flavus infection of developing corn.

Progress: Four test hybrids, each planted on three different dates (April, May, June), were grown in Georgia, Florida, Texas, South Carolina, Tennessee, Missouri, Ohio, Indiana, Illinois, Iowa, and Kansas. Larvae of European corn borer, corn earworm, and fall armyworm were collected at three sampling times (20, 40, and 60 days past silking) and individually evaluated for A. flavus infection. No significant variation in fungal infection of 1,621 larvae was associated with any of the factors in the experiment. A. flavus occurred on ca. 2 percent of insects regardless of variables. Populations of corn earworm were highest in Florida, South Carolina, and Tennessee; of European corn earworm in Illinois, Indiana, and Ohio; and of fall armyworm in Florida, Georgia, and South Carolina. These results will be related to insect damage on mature test ears and to aflatoxin contamination of their seed.

In associated limited studies, A. flavus was found to be present in leaf axils from the test corn in percentages ranging from 0 percent (Illinois, Tennessee) to 42 percent (South Carolina) (Iowa, 2.8%; Missouri, 25%; Texas, 30%; Kansas, 33%; Indiana, 40%); and European corn borer and corn earworm moths from a Missouri light trapping showed an A. flavus contamination rate of 48.7 percent and 59.6 percent, respectively.

c. Specific Objective: Characterize the time period for aflatoxin contamination of corn in the field at diverse locations.

Progress: Four test hybrids were grown in Georgia, Florida, Texas, Tennessee, Missouri, South Carolina, Kansas, Iowa, Ohio, Indiana, and Illinois. The time course of aflatoxin accumulation in untreated, preharvest corn was examined by harvesting test ears at 40 days post-silk, 60 days post-silk, and at maturity. Preliminary results demonstrate that aflatoxin is not present in samples from the Corn Belt. There was significant variation in southern corn between the levels of aflatoxin incidence in samples harvested at various times. For example, in Georgia corn there were no toxin-positive samples at the first harvest date but at maturity one-third contained detectable aflatoxin.

d. Specific Objective: Identify the role of European corn borer and corn earworm as vectors in transfer of A. flavus into the kernel region of developing ears.

Progress: Field experiments carried out in Georgia, Missouri, and Iowa included planting of a single hybrid at different times. Test ears were hand-infested with either European corn borer or corn earworm and some ears were inoculated with A. flavus by introducing a spore suspension into the silk bundle. At maturity, ears were evaluated for insect damage and sent to NRRC. Corn from these samples is currently being processed for aflatoxin assay.

Preliminary evaluation of data on the association of A. flavus with insects collected (3,104) at two sampling times for each planting date shows an overall 12.4-percent A. flavus infection rate in Georgia, 4.2 percent in Iowa, and 9.2 percent in Missouri. Significant differences

in A. flavus occurrence are associated with treatment, planting date, and sampling time in all states, but the pattern of these differences varies with the state. Higher infection percentages are associated with the later samplings of the May planting in all states.

Ears of corn from the 1975 crop obtained from an area of western Iowa experiencing preharvest aflatoxin contamination were examined for A. flavus. Although broad occurrence of the fungus was observed, distinct field-to-field variation existed. On specific test ears the fungus was invariably associated with kernel damage caused by feeding of larvae of the second generation European corn borer. A. flavus was detected only in damaged seed with no proliferation into adjoining undamaged kernels. The fungal profile on insect-damaged ears was generally restricted to three organisms; A. flavus, Penicillium oxalicum, and Fusarium moniliforme. Drought in western Iowa during July and August of 1975 appeared to be related to increased European corn borer damage of ears and A. flavus infection.

e. Specific Objective: Evaluate the mutagenic potential of foods and feeds naturally contaminated with mycotoxins.

Progress: The mutagenic potential of several mycotoxins was verified through utilization of the Salmonella typhimurium tester strains following the Ames procedure. However, experimental results were erratic. The factor responsible for the inconsistency appears to be the rat liver microsomal preparation. A practical method for determining the mutagenicity of crude preparations of agricultural commodities will require standardization of the microsomal component of the test system.

f. Specific Objective: Develop rapid screening method for zearalenone.

Progress: A rapid TLC method has been developed for zearalenone utilizing microslides coated with Adsorbosil 1. The plates were developed with chloroform:ethyl acetate:formic acid (6:3:1).

g. Specific Objective: Develop multitoxin analysis for penicillic acid, ochratoxin, and citrinin in grains.

Progress: Maximum absorption wavelengths of penicillic acid, citrinin, and ochratoxin were determined for HPLC. A TLC system was developed for citrinin in which the streaking of citrinin spots observed in published systems was prevented. Ion exchange chromatography for the purification of grain extracts containing citrinin was investigated, but recoveries of citrinin from columns was only 50 percent.

h. Specific Objective: Determine physical factors affecting mycotoxin analysis.

Progress: Coarsely ground corn samples received from USDA's Agricultural Marketing Service were separated into fractions based on particle size. Analyses of portions of the fraction from one sample containing large particles and chunks ranged from 5 to 76 p.p.b. The average level was 34 p.p.b. A grain sample must be finely ground and blended before a subsample is taken for analysis.

i. Specific Objective: Survey southern-grown grains and soybeans for aflatoxin, ochratoxin, and zearalenone.

Progress: Soybean samples (180) collected from the Southeast, South, and West did not contain aflatoxin, ochratoxin, or zearalenone. No aflatoxin or ochratoxin A was detected in wheat samples (102 lots) from Virginia, South Carolina, southeastern Missouri, southern Illinois, and Kentucky. Zearalenone was detected (0.36-11.05 p.p.m.) in 19 of 42 samples obtained in Virginia where there had been problems with mold damage of the wheat crop. Gibberella zeae was found in all zearalenone-positive samples. Four of 197 grain sorghum samples contained aflatoxin, and zearalenone was found in grain sorghum.

j. Specific Objective: Determine aflatoxin susceptibility of various soybean lines.

Progress: Fifteen soybean varieties were inoculated with five strains of aflatoxin-producing A. flavus and A. parasiticus. Differences in susceptibility to aflatoxin formation by the various soybean varieties were dependent on A. flavus or A. parasiticus strains used to inoculate soybeans.

k. Specific Objective: Detoxification of zearalenone.

Progress: Levels of zearalenone in corn grits and animal feed were reduced from 10 p.p.m. to <4 p.p.m. by treatment with vapors from paraformaldehyde for 7 days. Residual formaldehyde can be removed by aeration. The grits and feed were spiked with crystalline zearalenone. Zearalenone in solution is destroyed with formalin or sodium hypochlorite. The fluorescence of zearalenone disappeared in 0.5 N sodium hydroxide, but the reaction was reversed when alkaline solutions were neutralized. Propionic acid or sodium bicarbonate had no effect on zearalenone in solution.

l. Specific Objective: Find corn hybrids resistant to zearalenone.

Progress: A method has been developed to inoculate nonsterile corn with zearalenone producers to study corn hybrids and inbreds in an attempt to find a hybrid resistant to zearalenone formation. The solid substrate fermentation of 16 hybrids and inbreds inoculated with three different Fusarium graminearum strains has been studied. Little or no zearalenone was produced in two hybrids.

m. Specific Objective: Detoxification of vomitoxin-contaminated corn.

Progress: Preliminary data indicate the sulfur dioxide might react with vomitoxin. Toxicity of the reaction products has not yet been determined.

n. Specific Objective: Fermentative production of vomitoxin on solid substrate.

Progress: Vomitoxin has been produced on rice with yields of 100 mg./kg. grain. Higher yields are desirable.

o. Specific Objective: Develop an analytical assay for vomitoxin.

Progress: A preliminary method has been developed utilizing gas chromatography of TMS derivatives. This method is still under development. A more successful approach has been the initial experiments in use of coupled GC/MS with a programmed computor employing the principle of multiple ion detection. Detection and quantitation of vomitoxin and T-2 toxin resulted in samples of corn previously found negative by chemical analyses.

p. Specific Objective: Structure analysis of tremortin A.

Progress: Efforts by several cooperating laboratories to produce crystals adequate for x-ray analysis were unsuccessful. A quantity of tremortin A was sent to a cooperating English chemist for 300 mh NMR analysis.

q. Specific Objective: Clarify taxonomic relationships of the mycotoxin-producing Penicillia and the Fasciculata Section.

Progress: Penicillium expansum could be differentiated from P. crustosum by TLC of its metabolic products. This procedure enabled correction of some misidentified cultures.

r. Specific Objective: Dust control procedures for protection of personnel in handling mycotoxin-contaminated grain in large quantities will be developed.

Progress: Sources of dust emissions in the handling of contaminated corn on a large scale were found to be primarily when the grain was cleaned, being transferred from bin to bin, or being bagged. New grain cleaner, a dust collector to trap noxious dusts, and an incinerator for burning contaminated materials have been purchased and installed. The field site used for the large-scale detoxification of aflatoxin-contaminated corn was upgraded by the installation of bin stirrers, a dust collection and incineration system, safety ladders and platforms, the painting of bins, and other improvements in preparation for large-scale experiments in 1977.

s. Specific Objective: Investigate the nitrogen-containing compounds in the oil from ammoniated corn.

Progress: Samples of oil from ammoniated corn have undergone preliminary investigations to ascertain the functional form of nitrogen present. Amides of long-chain acids have been characterized, but the total amount of amides present does not account for all the bound nitrogen in the oil.

20840-006

Specific Objective: Determine ability of wild rice to support production of penicillic acid, zearalenone, and ochratoxin.

Progress: Wild rice at 45-percent moisture supported growth of A. ochraceus and produced 1 mg. ochratoxin A/g. rice. Negligible or nondetectable amounts of penicillic acid, vomitoxin, and zearalenone were synthesized under similar fermentation conditions with the appropriate fungus.

2. Progress Reports (Achievements):

20840-002

Publications:

CIEGLER, A., R. F. VESONDER, AND L. K. JACKSON. Production and Biological Activity of Patulin and Citrinin from Penicillium expansum. Appl. Microbiol., in press.

Other Reports:

CIEGLER, A. Patulin. Presented at Conference on Mycotoxins in Human and Animal Health, College Park, Maryland, October 4-8, 1976.

20840-003

Publications:

DeMARINI, D. M., C. P. KURTZMAN, D. I. FENNELL, K. A. WORDEN, AND R. W. DETROY. Transmission of PsV-F and PsV-S Mycoviruses During Conidiogenesis of Penicillium stoloniferum. J. Gen. Microbiol., in press.

DETROY, R. W. Mycoviruses: Significance in Industrially and Agriculturally Important Fungi. Microbiology 1976 (1976):563-564.

DETROY, R. W., P. E. STILL, AND D. DeMARINI. Fungal Metabolism and Double-Stranded RNA Viruses. Proc. 3rd International Congress of Virology, in press.

Other Reports:

DETROY, R. W. Propagation of Fungal Viruses: Use of Natural and Mutational Selection Methods. Presented at American Chemical Society meeting, San Francisco, California, August 29-September 3, 1976.

DETROY, R. W. Double-Stranded RNA Viruses from Fungi. Presented at Western Regional Research Center, Berkeley, California, September 2, 1976.

20840-005

Publications:

BECKWITH, A. C., R. F. VESONDER, AND A. CIEGLER. Chemical Methods Investigated for Detoxifying Aflatoxins in Foods and Feeds. Adv. Chem. Ser. 149 (1976):58-67.

BENNETT, G. A. Zearalenone Contamination in Corn Investigated. Baking Milling News 55 (1976):61-68.

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3. Plan of Work:

20840-002

a. Specific Objective: Characterize reaction products between patulin and cysteine and glutathione. Determine toxicity of adducts formed.

Plan of Work: Conduct reactions at low temperatures with controlled pH, time, and reactant concentrations to minimize number of adducts formed and degradation. Isolate products at low temperature and determine toxicity to the chick embryo.

b. Specific Objective: Characterize product formed between citrinin and corn. Determine factors involved in adduct formation and toxicity of isolated substances to chick embryo and mice.

Plan of Work: Grow Penicillium citrinum on corn and isolate adduct by conventional means. Determine factors such as moisture level, pH, and concentration in adduct formation. Test adducts formed in chick embryo and mice.

c. Specific Objective: Determine reasons for "disappearance" of ochratoxin in contaminated corn and toxicity of corn after ochratoxin can no longer be detected.

Plan of Work: Naturally contaminated corn in which high levels of ochratoxin had been detected and which no longer analyze positively for this toxin will be extracted and the extract tested for toxicity in chick embryo. Fractionation of lethal extracts by conventional means will be used to isolate toxins. Radiolabelling will be applied if results warrant further research.

20840-003

a. Specific Objective: Determine nutritional requirements for optimum mycovirus production in fungal tissue.

Plan of Work: Optimum nutritional conditions of N, C, and aeration will be determined to yield maximum fungal growth and/or virus production. Various carbon, nitrogen, protein, and amino acid sources will be examined to measure fungal growth-virus ratios. Tests will be evaluated to determine what environmental conditions either promote or perturb virus multiplication.

b. Specific Objective: Determine mycotoxin-mycovirus interactions.

Plan of Work: Investigate the mode of virus replication in P. stoloniferum tissue using radioactively labeled precursors for viral double-stranded RNA and protein coat of the virus. Specific fungal metabolites, i.e., mycotoxins, antibiotics, and antivirals will be tested using an in vivo virus multiplication system in P. stoloniferum.

c. Specific Objective: Examine insect-pathogenic and plant-pathogenic fungi for viruses.

Plan of Work: A standardized screen will be utilized to test economically important insect and plant-pathogenic fungi for viruses. Specific buffer-extraction techniques will be employed to determine the presence of viruses in fungal tissue and/or conidia, coupled with transmission electron microscopy, differential centrifugation, and gel electrophoresis.

20840-005

a. Specific Objective: Develop analytical methods (screening and quantitative) for zearalenone that can be used on different commodities and for other mycotoxins.

Plan of Work: Two-dimensional and multiple development TLC will be investigated as well as high pressure liquid chromatography to measure quantities of zearalenone and other mycotoxins. Solvent used to extract zearalenone from commodities and methods for purification of extracts will be studied.

b. Specific Objective: Survey wheat and corn from specific regions in the Southeast to determine how incidences of mycotoxins (e.g., zearalenone and aflatoxin) change from year to year and how climatic conditions affect mycotoxin outbreaks. Survey grain sorghum for mycotoxins.

Plan of Work: Wheat and corn samples will be collected from different counties of Virginia, including counties where zearalenone was a problem in the 1975 wheat crop. Samples will be tested for zearalenone, aflatoxin, and ochratoxin, and results will be compared to those obtained in previous years and mycotoxin incidences from counties with different weather conditions will be compared. Grain sorghum samples will be analyzed from different parts of the country.

c. Specific Objective: Survey corn for sterigmatocystin and study the stability of sterigmatocystin in corn spiked with the mycotoxin and naturally contaminated corn.

Plan of Work: Corn samples will be tested for sterigmatocystin. A solid substrate fermentation will be used to obtain levels of sterigmatocystin in corn that might be typical of a natural contamination. The fermented corn will be used in the study of stability of sterigmatocystin in corn.

d. Specific Objective: Find corn hybrids resistant to zearalenone formation and contamination and to study the formation of zearalenone in field corn.

Plan of Work: Nonsterile corn hybrids and inbreds will be inoculated by toxin-producing strains of fungi and fermented by the method developed in the laboratory. Levels of zearalenone will be determined in the fermented corn and the degree of resistance to zearalenone formation of hybrids and inbreds will be used to decide which genetic material might be desirable in a hybrid. In the field, the same hybrids and inbreds will be inoculated with the fungi. The corn will then be studied by plant pathologists to determine the effect of the fungi on the plant. Corn from the plants will be tested for zearalenone.

e. Specific Objective: Develop multitoxin analysis for penicillic acid, ochratoxin, and citrinin in grains.

Plan of Work: HPLC studies of the three mycotoxins will be continued to determine lower limits of detection, linearity, and reproducibility of injections. TLC studies will be completed and will include effects of contaminants in grain extracts upon fluorescence of added citrinin. Methods of extraction and purification will be investigated to develop a quantitative procedure.

f. Specific Objective: Develop rapid quantitative method for aflatoxin M_1 in dairy products.

Plan of Work: Current AOAC cellulose column chromatography will be studied to reduce size and solvent volumes necessary to obtain clean extracts. A method will be developed that requires less time and less toxic solvents and is more reliable than accepted methods. A more sensitive chemical confirmatory test for M_1 will be developed. Comparisons of the quantitative method and chemical confirmatory test with accepted AOAC methods will be made.

g. Specific Objective: Study distribution of mycotoxins, particularly aflatoxin and zearalenone, within contaminated lots of corn to assist in both laboratory and large-scale detoxification studies.

Plan of Work: Determine variations between aflatoxin or zearalenone levels in various size samples from the same lot of corn to be used in detoxification experiments. Determine what percentage of the kernels contain aflatoxin in highly contaminated lots of corn to be blended for feeding and detoxification studies.

h. Specific Objective: Study the stability of mycotoxins in naturally contaminated and spiked corn during storage to determine possible changes in levels that would affect long-term investigations.

Plan of Work: Determine possible decreases or increases in mycotoxin levels in cereal grains during short-term (weeks) and long-term (months or years) storage. Both naturally contaminated cereal grains and grains spiked at several levels of mycotoxin would be analyzed after short-term storage. Then a long-term storage experiment would be started.

i. Specific Objective: Detoxification of zearalenone.

Plan of Work: The chemistry of the reaction between zearalenone and paraformaldehyde leading to toxin destruction will be studied, and reaction products will be characterized. Conditions for the detoxification of zearalenone in corn will be maximized. Animal feeding tests will be conducted to see whether zearalenone contaminated corn.

j. Specific Objective: Zearalenone in products from corn oil refining will be determined.

Plan of Work: Analytical methods for determining zearalenone in products will be developed first on products spiked with zearalenone. Then methods will be applied to products obtained from several corn lots naturally contaminated with zearalenone.

k. Specific Objective: Continue the investigation of nitrogen-containing compounds in the oil from ammoniated corn.

Plan of Work: Separation, isolation, and characterization of the components in the polar fraction of oil by chromatographic and spectroscopic means.

1. Specific Objective: Initiate study of the chemistry of aflatoxin as influenced by corn constituents.

Plan of Work: Proposed interactions of aflatoxin with corn constituents will be studied by fractionation of aflatoxin-contaminated corn preparation.

m. Specific Objective: Produce an increased aflatoxin content in contaminated corn on a large scale for use in cattle-feeding trials.

Plan of Work: The molds present on aflatoxin-contaminated corn will be induced to produce a high level of aflatoxin on the host corn for decontamination studies. Conditions of temperature, time, moisture level, and humidity will be established for the production of aflatoxin on large volumes of corn in a field situation. Safety and health hazards will be examined and minimized.

n. Specific Objective: Detoxify 800 bushels of aflatoxin-contaminated corn for use in cattle-feeding trials.

Plan of Work: Eight-hundred bushels of aflatoxin-contaminated corn will be detoxified using the ammoniation process developed at NRRC. The treated corn will be fed to cattle to fulfill FDA requirements for ultimate FDA approval for the process.

o. Specific Objective: Complete studies on roasting corn as a means of lowering aflatoxin content.

Plan of Work: Obtain the use of a commercial (Jo-Fran) farm-type roaster and conduct a series of tests to determine suitability of the machine to reduce aflatoxin content of contaminated corn and the conditions of temperature, moisture, and humidity optimum for decontamination.

p. Specific Objective: Examine untested hybrid corn lines for genetic characteristics of resistance to A. flavus infection during seed development.

Plan of Work: Complete sets of single cross hybrids and their parental inbreds will be planted at locations in the southern U.S. and Midwest. Developing ears will be inoculated with A. flavus spores to provide the appropriate challenge for determining resistance.

q. Specific Objective: Assess the potential for development of hybrid corn lines incorporating several characteristics of resistance to insect and fungal attack.

Plan of Work: Hybrids will be developed that simultaneously possess high levels of DIMBOA or similar insect resistance characters, tight husks or thick pericarps providing physical resistance to predation and unique characteristics such as anthocyanins in the aleurone that appear to provide some protection from fungal invasion. Hybrids will be grown in the southern U.S. where the natural occurrence of aflatoxin in control corn provides a basis for determining resistance in test varieties without the imposition of simulated inoculation.

r. Specific Objective: Determine the inoculum source of toxin-producing fungi in barley.

Plan of Work: In a cooperative study with Scandinavian scientists, areas of Denmark identified as the source of barley heavily contaminated with ochratoxin will be defined. Within these regions, plant parts and seed of the developing crops will be examined for the presence of Aspergillus and Penicillium species. Individual fungal isolates will be tested for their ability to produce ochratoxin. Occurrence of the toxigenic fungi will also be studied during the harvest and in the immediate postharvest period.

s. Specific Objective: Study the reason for the predominance of A. flavus over A. parasiticus in preharvest corn contaminated with aflatoxin.

Plan of Work: Developing corn will be inoculated with various ratios of A. flavus and A. parasiticus spores. Competitive ability of the two species in establishing infection and producing aflatoxin will be measured by determining the level of the four primary aflatoxins, B_1, B_2, G_1, and G_2, in mature corn. Since A. flavus produces only B_1 and B_2 and A. parasiticus synthesizes all four toxins, the ratio of B_1 to G_1 provides an estimate of the development of the two microbes.

t. Specific Objective: Develop analytical methods for the trichothecene family of mycotoxins and determine trichothecenes present in naturally contaminated grains.

Plan of Work: Gas chromatography of TMS derivatives coupled with a programmed computer will form the basis of the method. Various procedures will be explored for the most efficient extraction of trichothecenes from naturally contaminated corn and wheat. Extracts will be analyzed for trichothecenes by TLC, GC, and MID.

u. Specific Objective: Develop solid substrate fermentations for production of trichothecenes.

Plan of Work: Various fermentation parameters will be explored to optimize production of trichothecenes on grains.

v. Specific Objective: Determine dosages of vomitoxin required to ellicit refusal and vomiting in swine and to determine pharmacological action in laboratory animals.

Plan of Work: Sufficient vomitoxin will be produced by fermentation to carry out cooperative studies at the Universities of Illinois and Mississippi.

v. Specific Objective: Develop procedures for production, purification, and analysis of moniliformin; determine its toxicity to laboratory animals.

Plan of Work: Standard procedures of fermentation, recovery, and purification will be followed. Toxicity will be tested in mice and the chick embryo.

B. Summary of Progress for Extramural Projects:

1. Progress Report (Narrative):

8004-20840-008

Corn samples (191) were collected following the crop year 1975 from terminal elevators and silos at feed-processing establishments in several regions in Yugoslavia and in the region Posavina where the "Balkan kidney disease"--endemic nephritis--is widespread. Samples were analyzed for zearalenone, aflatoxins, skin-irritating factors, and ochratoxin A by a modified Eppley method. Aflatoxins were not detected in any of the corn samples. Ochratoxin A was reported in 26 percent of the samples in levels ranging from 0.045-5.1 ppm and averaging 0.49 ppm. Zearalenone was found in 2.6 percent of the samples

in levels ranging from 0.043-10.0 ppm and averaging 5.10. These levels are higher than those observed in surveys of marketable corn in the United States. The most important finding was that 8.3 percent of the corn samples had a skin-irritating factor or factors indicating a possible presence of trichothecenes. Levels of the equivalent of 0.5-5.0 ppm. T-2 toxin were observed with an average level of 2.2 ppm. The highest incidence (28%) and highest levels (>5 ppm) of skin-irritating factors occurred in samples collected from the region (Posavina) where endemic nephritis is widespread.

2. Progress Reports (Achievements):

Publications:

BOGDANIC, C., S. MUZIC, AND I. BALZER. Mycotoxins in Livestock Fodder. Works of the Agricultural Faculty, University Sarajevo. XXIV, 27 (1976):519-530.

MUZIC, S., C. BOGDANIC, AND I. BALZER. Establishment of Toxic Metabolites of Certain Moulds in Livestock Fodder. Works of the Agricultural Faculty, University Sarajevo. XXIV, 27 (1976):531-538.

Other Reports:

BALZER, I. Natural Contamination of Corn (Zea mais) with Mycotoxins. Presented at III International Union of Pure and Applied Chemistry Symposium on Mycotoxins in Foodstuffs, Paris, France, September 16-18, 1976.

FOOD AND NUTRITION RESEARCH

CRIS Work Units Listed by Work Reporting Unit

Work Reporting Unit (WRU)

Number	Title (Principal Investigator)
FOOD COMPOSITION AND IMPROVEMENT (p. 162)	
3102-20900-001	Enzyme modification of wheat flour to improve nutritional quality (H. W. Gardner)
3102-20900-002	Composition and properties of cereal grain fibers for foods (F. R. Dintzis)
8003-20900-013	Evaluation of soybean protein isolates and concentrates as meat additives (W. J. Wolf)
HUMAN REQUIREMENTS FOR NUTRIENTS (p. 166)	
3102-20910-001	Biological discrimination among unsaturated fatty acid isomers (T. L. Mounts)
3102-20910-002	Preparation of labeled isomeric fats for human ingestion and analysis of their metabolic products (E. A. Emken)
3102-20910-003	Biological utilization of isomeric fatty acids from hydrogenated soybean oil (E. A. Emken)

FOOD COMPOSITION AND IMPROVEMENT

A. Technological Objective: To provide accurate up-to-date and comprehensive information in a readily usable form on the composition of all important foods for those nutrients required by and biologically useful to man.

1. Progress Report (Narrative):

20900-001

a. Specific Objective: To improve the storage and safety of foods by understanding the properties and products of fat oxidation by both enzymic and nonenzymic means.

Progress: We have found that soybean extracts degrade linoleic acid and 13-hydroperoxylinoleic acid into numerous products, including 11-hydroxy-trans-12,13-epoxy-cis-9-octadecenoic, 9-oxo-trans-12,13-epoxy-trans-10-octadecenoic, 9-hydroperoxy-trans-12,13-epoxy-trans-10-octadecenoic, 13-oxooctadeca-9,11-dienoic, and 13-hydroxyoctadeca-9,11-dienoic acid. These products are precisely duplicated by a model reaction where 13-hydroperoxylinoleic acid was degraded by ferric chloride and cysteine. Thus, soybeans appear to be degrading fat hydroperoxides by a mechanism quite different from that observed previously in cereals. These differences in mechanism undoubtedly have an effect on the flavor differences between soy and cereals.

b. Specific Objective: To develop new engineered foods composed of starch, xanthan gum, and other food ingredients.

Progress: Breads were developed that were composed primarily of starch, vegetable protein (peanut or cottonseed), and gums (xanthan or xanthan-guar combination). The rheology of various combinations of the above ingredients are being studied to detect unique interactions and effects.

20900-002

a. Specific Objective: To determine factors that influence binding of radioactive iron to dietary fiber.

Progress: Effects of iron concentration, temperature, and time of exposure to iron containing solutions have been measured for two wheat bran mill fractions.

b. Specific Objective: To provide dietary fiber for feeding studies carried out at the Human Nutrition Laboratory (HNL) as part of a cooperative effort to determine effects of dietary fiber incorporated into controlled human diets and to analyze dietary fiber in resulting fecal samples.

Progress: Roughages such as wheat and corn bran are being retrieved from human feces and composition determined in terms of defined components of plant tissues such as cellulose, hemicellulose, ash, oil, and starch.

2. Progress Reports (Achievements):

20900-001

Publications:

GARDNER, H. W. AND D. J. SESSA. Degradation of Fatty Acid Hydroperoxide by Cereals and a Legume: A Comparison. Proceedings, 13th World Congress, International Society for Fat Research, Marseilles, France (in press).

SESSA, D. J., H. W. GARDNER, R. KLEIMAN, AND D. WEISLEDER. Oxygenated Fatty Acids from Soybean Phosphatidylcholines and Their Possible Derivation from Hydroperoxides. Proceedings, 13th World Congress, International Society for Fat Research, Marseilles, France (in press).

SESSA, D. J., H. W. GARDNER, R. KLEIMAN, AND D. WEISLEDER. Oxygenated Fatty Acid Constituents of Soybean Phosphatidylcholines. Lipids (in press).

Other Reports:

GARDNER, H. W., R. KLEIMAN, AND D. WEISLEDER. Lipid Hydroperoxide Degradation by a Model System. Presented at AOCS Fall Meeting, Chicago, Illinois, September 26-29, 1976.

GARDNER, H. W. AND D. J. SESSA. Degradation of Fatty Acid Hydroperoxide by Cereals and a Legume: A Comparison. Invited Symposium at 13th World Congress, International Society for Fat Research, Marseilles, France, August 30-September 4, 1976.

SESSA, D. J., H. W. GARDNER, R. KLEIMAN, AND D. WEISLEDER. Oxygenated Fatty Acids from Soybean Phosphatidylcholines and Their Possible Derivation from Hydroperoxides. Invited Symposium at 13th World Congress, International Society for Fat Research, Marseilles, France, August 30-September 4, 1976.

GARDNER, H. W. Degradation of Lipid Peroxides. Invited Seminar, Eastern Regional Research Center, Philadelphia, Pennsylvania, February 1, 1977.

ANON. Food Production: Bread-Making Method. Technol. Mart 6, No. 4 (1976).

ANON. New Food Research. Foods and Home Notes, USDA, No. 20, May 17, 1976, p. 3.

3. Plan of Work:

20900-002

a. Specific Objective: Examination of factors influencing iron binding of wheat bran will continue in order to find methods to reduce potential loss of availability of minerals in human dietary metabolism.

Plan of Work: Effects of variables such as particle size, phosphorous content, and baking upon the iron binding ability of wheat bran will be measured by radioactive tracer methods.

b. Specific Objective: Cooperation with Human Nutrition Laboratory will continue as dietary roughages will be supplied and examined in order to correlate effects of dietary fiber type and composition with diet and physiological effects upon humans.

Plan of Work: This laboratory will supply characterized roughages to HNL as required. Retrieval of dietary fiber materials from feces and examination of composition by the Van Soest ADF or NDF methods and by gas chromatography will be done to determine how dietary fibers are altered by passage through the human digestive tract. (See also 3102-20520-023 under "Technologies for Food and Feed Uses of Field Crops.")

B. Summary of Progress for Extramural Projects:

1. Progress Report (Narrative):

8003-20900-013

Functional properties were evaluated of ground meat-soy protein mixtures containing 10-50% soy protein in the form of soy grits, textured flour, concentrate, or isolate. Increasing the amount of soy protein caused decreases in viscosity and

water retention plus losses of desirable texture and flavor. Loss of desired flavor was caused both by dilution of the meat flavor and the residual flavors of soy. Isolate and textured flours could be added up to 20%, concentrate up to 30%, and grits up to 10% before flavor scores became unacceptable for the meat-soy mixtures. Preliminary studies have been made on the effects of the carbohyrates in soy flours and concentrates on viscosity, water absorption, foaming ability, and foam stability. Substitution of 20% of the meat with soy flakes in a canned meat product caused some lowering of net protein utilization and protein efficiency but the values were still above those obtained for a casein control.

HUMAN REQUIREMENTS FOR NUTRIENTS

A_1 Technological Objective: Develop recommendations for lipid uptake by humans and identification of the useful nutrient forms to meet the recommendations.

1. Progress Report (Narrative):

20910-001

a. Specific Objective: Develop synthetic methods and begin preparation of radioisotope labeled polyunsaturated fatty acid isomers.

Progress: Selective reduction of naturally occurring cis-9-octadecen-12-ynoic acid using poisoned Lindlar catalyst and tritiated water yielded 9-cis,12-cis-octadecadienoic acid-12(13)-^{3}H. A procedure for isomerization using nitrous acid was used to obtain 9-trans,12-trans octadecadienoic acids from the cis,cis isomers containing carbon-14 and tritium. The metabolism of these tagged isomers will be studied in the laying hen.

b. Specific Objective: Begin feeding experiments involving carbon-14 and tritium labeled polyunsaturated fatty acids and finish feeding experiments involving trans positional octadecenoic acid isomers.

Progress: Dual-labeled radioactive mixtures composed of (1) 9-trans,12-trans octadecadienoate-12(13)-^{3}H (linoelaidate-12(13)-^{3}H) and 9-cis,12-cis linoleate-1-^{14}C, (2) linoelaidate-1-^{14}C and linoleate-12(13)-^{3}H and (3) linoleate-12(13)-^{3}H and linoleate-1-^{14}C were each fed to three laying hens. Analyses of egg yolk neutral lipid and phospholipid fractions showed no variation in the dienoic ^{3}H/^{14}C ratios from the ratios fed. Detailed analysis of individual neutral lipid components indicated: (1) discrimination against the trans,trans isomer in cholesterol esters; (2) no discrimination against either isomer in triglycerides; and (3) loss of ^{3}H during synthesis of cholesterol. Elongation of dienoic acid to arachidonic acid was restricted to the cis,cis isomer and occurred only in the phospholipid fraction.

Project terminated November 29, 1976. Work continued in part under 20910-003.

20910-002

a. Specific Objective: Compare metabolism in humans of deuterated Δ8 and Δ10 monounsaturated fatty acid isomers.

Progress: The cis and trans triglycerides of deuterated cis- and trans-Δ8 and Δ10 octadecenoic acids have been prepared and are ready for feeding to human volunteers. A triple-labeled experimental design which involves feeding three differently labeled fatty acid isomers allowed both the cis and trans isomers of octadecenoic acid to be simultaneously compared to each other and to oleic acid. In addition, the total number of future feeding studies will be reduced by 50% with a corresponding reduction in the total number of samples that need to be fractionated, derivitized, and analyzed by GC-MS. The feasibility of this novel approach has been demonstrated in a study using O1-d_2, E1-d_4, and O1-d_6.

b. Specific Objective: Synthesis of deuterated cis and trans Δ11, Δ12, and Δ13 monounsaturated fatty acid isomers.

Progress: Sixteen deuterated cis and trans positional monounsaturated fatty acid isomers have been synthesized in sufficient quantities for use in human feeding studies. The triglycerides of the labeled fatty acid isomers have been prepared: 8c-18:1-12,12,13,13-d_4; 8t-18:1-12,12,13,13-d_4; 10c-18:1-14,14,15,15-d_4; 10t-18:1-14,14,15,15-d_4; 11c-18:1-10,10,15,15,16,16-d_6; 11t-18:1-15,15,16,16-d_4; 11c-18:1-15,16-d_2; 12c-18:1-9,10,15,15,16,16-d_6; 12t-18:1-15,15,16,16-d_4; 12c-18:1-15,15,16,16-d_4; 12t-18:1-9,10-d_2; 12c-18:1-9,10,15,15,16,16-d_6; 13c-18:1-17,17,18,18-d_4; 13t-18:;-17,17,18,18-d_4; 13c-18:1-17,18-d_2, 13t-18:1-17,18-d_2. In addition, enough of the control fatty acid, 9c-18:1-14,14,15,15,17,18-d_6, has been prepared for six feeding experiments.

These fatty acid isomers represent the five most prevalent monounsaturated fatty acids found in hydrogenated soybean oil. Only 8c-18:1-d_2, 8t-18:1-d_2, 10c-18:1-d_2, and 10t-18:1-d_2 still remain to be synthesized in order to complete this phase of the synthetic work. An outgrowth of this research has been the improvement of yields from the Wittig reaction from 40 to 70%.

c. Specific Objective: Total synthesis of deuterated octadecadienoic acid isomers.

Progress: The synthesis of specifically deuterated polyunsaturated isomers has started. Potential synthetic routes are being explored to determine which are the most promising. A silver-resin chromatographic procedure has been developed which can separate and purify the c,c-, c,t-, and t,t-geometric isomers of octadecadienoic acids. The development of this methodology was a necessary step before a final synthetic sequence for preparation of the positional octadecadienoic acids could be devised.

2. Progress Reports (Achievements):

20910-001

Publications:

LANSER, A. C., T. L. MOUNTS, AND E. A. EMKEN. Metabolism of Linoleate versus Linoelaidate in the Laying Hen. J. Am. Oil Chem. Soc. 53 (1976):468A. Program Abstract.

MOUNTS, T. L. Double Bond Position Affects Metabolism of cis-Octadecenoates. Lipids 11 (1976):676-679.

Other Reports:

LANSER, A. C., T. L. MOUNTS, AND E. A. EMKEN. Metabolism of Linoleate versus Linoelaidate in the Laying Hen. AOCS Fall Meeting, Chicago, Illinois, September 1976.

20910-002

Publications:

DEJARLAIS, W. J. AND E. A. EMKEN. Syntheses of Tetra- and Hexadeuterated Octadecenoates. Lipids 11 (1976):594.

3. Plan of Work:

20910-002

a. Specific Objective: Compare metabolism in humans of 12,cis- and 12,trans-octadecenoic acids to oleic acid. This work will provide basic information necessary for determining if the isomeric fats formed during hydrogenation

of soybean oil have an impact on the fatty acid structure of plasma, lipoprotein, and membrane lipids in man.

Plan of Work: The advantages of our successfully implemented triple-labeled experimental design in humans mandates that this procedure be used in all remaining experiments. The 12,cis- and 12-trans-octadecenoic acids are prominent isomers formed during hydrogenation of linolenate and linoleate in soybean oil and will be the next isomers investigated. The procedure will be to feed 9c-18:1-9,10-d_2, 12t-18:1-15,15,16,16-d_4, and 12c-18:1-9,10,15,15,16,16-d_6 to one subject and 9c-18:1-14,14,15,15,17,18-d_6, 12t-18:1-9,10-d_2, 12c-18:1-15,15,16,16-d_4 to a second subject. These experiments vary the number and position of the deuterium label and will allow detection of possible deuterium isotope effects. Blood samples will be taken and the lipoprotein, plasma, and red cell lipids fractionated into various individual neutral and phospholipid components.

b. Specific Objective: Develop a practical synthetic method which can be used for preparing linoleic acid-d_6 for use in human metabolism experiments.

Plan of Work: The homogeneous catalyst methyl benzoate chromium tricarbonyl, will be investigated as a method for introducing deuterium in conjugated en-yn alkyl halides. Reduction of conjugated en-yn systems are not possible with the catalyst we are currently using, but this step is vital for the practical preparation of linoleic acid-d_6.

c. Specific Objective: Increase the number of fatty acid isomers which can be studied in human subjects per year.

Plan of Work: Key personnel previously associated with Georgetown University Medical School and the isomeric fatty acid metabolism studies in humans have left Georgetown's clinical study unit. This has resulted in a long delay in scheduling new studies. The possibility of moving our cooperative research program to the Peoria School of Medicine will be explored with the anticipation that the proximity and better logistics will facilitate scheduling experiments. Installation of additional mass spectroscopy equipment should permit handling the increased analytical load which will result if more experiments can be scheduled.

20910-003

a. Specific Objective: Complete acyl-positional analyses of phospholipids and triglycerides from feeding experiments involving carbon-14 and tritium labeled trans,trans- and cis,cis-isomers of the essential fatty acid (linoleic acid).

Plan of Work: Individual phospholipid components will be isolated by preparative thin-layer chromatography and analyzed by both scintillation counting and combined gas-liquid chromatography-liquid scintillation counting. Enzymatic cleavage of individual lipid components will be used to determine if the radiochemically tagged fatty acid isomers are incorporated into specific acyl positions of triglycerides and phospholipids.

b. Specific Objective: Prepare tritium labeled trans-8,10 and 11 isomers of oleic acid for use in feeding experiments with laying hens.

Plan of Work: Tritium labeled 8,10 and 11-trans octadecenoic acids will be synthesized by nitrous acid isomerization of their corresponding cis isomers. Radiochemically and chemically pure acids will be obtained by preparative thin-layer chromatography as necessary.

c. Specific Objective: Analyze individual egg lipid components from 12-trans octadecenoic acid feeding experiments.

Plan of Work: Egg neutral lipids and phospholipids from 12t-18:1 feeding experiments will be isolated by preparative thin-layer chromatography. Analysis of each component by scintillation counting and combined gas-liquid chromatography-liquid scintillation counting. Enzymatic cleavage will be used to determine incorporation of radiochemically tagged fatty acids into specific acyl positions of neutral and phospholipid components.

PRODUCTION EFFICIENCY RESEARCH

CRIS Work Units Listed by Work Reporting Unit

Work Reporting Unit (WRU)

Number	Title (Principal Investigator)
INTRODUCTION, CLASSIFICATION, MAINTENANCE, EVALUATION, AND DOCUMENTATION OF PLANT GERM PLASM (p. 173)	
3102-20160-001	Characterization of selected unusual seed components (C. R. Smith)
3102-20160-002	Anti-tumor agents from plants (C. R. Smith)
3102-20160-003	Chemical survey of uncultivated plants for valuable seed components (R. Kleiman)
3102-20160-004-A	Improved Brassica cultivars as potential domestic sources of high-erucic oil (R. Kleiman)
3102-20160-005	Natural pest control agents from seeds and potential new crops (B. Freedman)
3102-20160-006	Analysis and processing of Papaver somniferum (H. L. Tookey)
3102-20160-007-A	Genetic improvement and agronomic potential of promising new industrial crops (K. D. Carlson)
8002-20160-228	Essential oils from Umbelliferae plants in Pakistan (R. Kleiman)
8001-20160-236	Survey of Indian herbaceous plants for a new source of seed oils (G. F. Spencer)
8001-20160-244	Seed sources of petroselinic and conjugated trienoid acids (L. H. Princen)
8001-20160-245	Use of plant tissue culture for preparing useful products (N. E. Delfel)
PHYSIOLOGICAL AND BIOCHEMICAL TECHNOLOGY TO IMPROVE CROP PRODUCTION (p. 185)	
3102-20170-001	Increased photosynthetic efficiency of plants through yellow chloroplast pigments (H. J. Dutton)
3102-20170-002	Relationships of nitrogen fixation in microorganisms and plants to reduce energy requirements of crops (J. W. Newton)
3102-20170-003	Plant cell and tissue culture for the bioproduction of valuable chemicals (N. E. Delfel and J. A. Rothfus)

BIOLOGICAL AGENTS FOR PEST CONTROL (p. 195)

3102-20260-001	Biochemistry of toxin production in Bacillus thuringiensis (D. E. Johnson)

AGRICULTURAL CHEMICALS TECHNOLOGY FOR CROP PROTECTION AND MODIFICATION (p. 202)

3102-20290-001	Chemistry of novel plant growth promoting agents (brassins) (M. D. Grove)

STRUCTURES, EQUIPMENT, AND SYSTEMS FOR LIVESTOCK PRODUCTION (p. 204)

3090-20401-001-C	Pilot plant production of methane by digestion of feedlot waste (R. A. Rhodes)

CONTROL OF CATTLE DISEASES (p. 205)

3102-20420-001-A	Bioassay in cattle of chemical fractions from toxic tall fescue forage (S. G. Yates)
3102-20420-002	Toxic principles of tall fescue (H. L. Tookey)

INTRODUCTION, CLASSIFICATION, MAINTENANCE, EVALUATION, AND DOCUMENTATION OF PLANT GERM PLASM

A. Technological Objective: New and improved knowledge of the chemical, biological, and agronomic potentials of selected plant species as new crop sources of industrial oils, waxes, gums, fibers, of food and feed proteins, and licit and illicit narcotic drugs and other medicinals.

1. Progress Report (Narrative):

20160-001

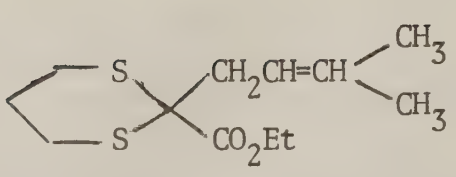
a. Specific Objective: Characterize and evaluate potential usefulness and biochemical significance of novel constituents in plant seeds.

Progress: The principal triglyceride component of Polygala virgata (family Polygalaceae) seed oil is a unique 2-acetotriglyceride. The seed oil of another polygalaceous species, Securidaca longipedunculata, contains a complex mixture of triglycerides with estolide linkages that incorporate conjugated dienols. Securidaca seed oil yields two new homologs of coriolic acid: 11-hydroxyhexadeca-cis-7, trans-9-dienoic acid, and 9-hydroxytetradeca-cis-5, trans-7-dienoic acid. The phenolic fraction of Rapanea laetevireus (family Myrsinaceae) seed oil is comprised of series of 5-alkylresorcinol with various sidechains, including those that are C_{11} and C_{13} saturated as well as C_{11}, C_{13}, and C_{15} monoenoic. Salvia plebeia yields a unique lignan diester which, upon hydrolysis, yields ferulic acid, (+)-12L-methyltetradecanoic acid and secoisolariciresinol.

20160-002

a. Specific Objective: Synthesis of additional readily prepared esters of cephalotaxine for bioassay.

Progress: Developments early in the year occasioned a shift in synthetic priorities so that emphasis has been placed on conversion of cephalotaxine to one of its naturally occurring active esters. Methods have been developed for preparation of the following compound, a potential intermediate in the transformation of cephalotaxine to harringtonine:



b. Specific Objective: Preparation of new plant extracts for bioassay.

Progress: One hundred and fifty-two additional extracts have been prepared and submitted for bioassay during this reporting period. New actives are as follows: Trevia multiflora and Euphorbia lagascae are active in the P388 murine leukemia system; Anthrosomanea vesiculosum and Mallotus albus show cytotoxic activity only.

c. Specific Objective: Fractionation of "confirmed actives."

Progress: From half a ton of seed, two compounds have been isolated which probably represent the antileukemic principle of Sesbania drummondii. The active principle of Cephalotaxus mannii has been enriched 500 fold. Fractionation of the following actives is in progress: Annona glauca, Anthrosomanea polyantha, Daphne pontica, Datisca cannabina, Erythrophleum guineense, Nerium indicum, Tephrosia nyikensis, Thevetia thevetioides.

20160-003

a. Specific Objective: Discover and characterize novel plant seed oils.

Progress: Seed from 69 uncultivated plant species were chemically screened. Protein contents ranged from 5 to 47% and oil percent from 4 to 49%. Spectroscopic and chromatographic analyses indicated a variety of functional groups, including hydroxyl, conjugated and epoxy groups. Gas chromatography, coupled with mass spectrometry, detected and identified several unusual fatty acids: (1) a series of new omega-5 monoenoic acids in Grevillea robusta of chainlengths C_{14} to C_{28} totalling 23% of the seed oil, (2) 62% of Wrightia tincloria is 9-hydroxy-cis-12-octadecenoic acid, and (3) lesquerolic acid (21%) was found in Heliophila amplexicaulis.

Characterization of two oils showed the presence of (-)-(S,S)-12-hydroxy-13-octadec-cis-9-enolide, a 14-member lactone in Crepis conyzaefolia seed oil and a secoisolariciresinol branched-chain fatty diester from Salvia plebia oil.

b. Specific Objective: Provide compositional data in support of plant breeding and agronomic research.

Progress: In cooperation with plant breeders, 32 winged bean samples were analyzed for oil and protein content and fatty acid composition. These same analyses were performed on 21 Stokes' Aster samples, 24 okra samples, and 15 varieties of filberts. In addition to these tests, glucosinolate content was also found for 32 Limnanthes and 490 Brassica samples. Ninety-eight Crambe accessions were analyzed for oil and protein. Seeds were grown in such diverse locations as Corvallis, Oregon, and Mayaguez, Puerto Rico.

20160-004A

a. Specific Objective: To develop seeds of Brassica cultivars with low glucosinolate content and high erucic acid content.

Progress: The best winter annual, cold-tolerant Brassica napus seed contained 44% oil, 54% erucic acid, and 1.7% glucosinolates. Seed from the best summer type B. campestris contained 39% oil, 62% erucic acid, and 4.4% glucosinolates.

In seed yield trials winter annual, cold-tolerant B. campestris yielded 1,973 kg/ha, summer annual B. campestris yielded 593 kg/ha and spring planted B. carinata yielded 1,365 kg/ha.

20160-005

a. Specific Objective: Discover and characterize useful pesticidal activity in plant seed.

Progress: Pesticidal activities have been tentatively detected in seeds of the following plants: Crysanthemum segetum and Tragia incana (morphogenic activity against Indian meal moth); Tragia incana, Zanthoxylum acanthopium, and Malva aegyptia (repellent activity against confused flour beetle); Trevia nudiflora (active against poultry lice); Bassia hyssopifolium, Antidesma nigricans, Apium sellowianum, Asparagus racemosus, Asphodelus albus, Berrya ammonilla, Bonjeania recta, Bupleurum fontansii, Butea frondosa, and Clarkia amoena (active against European corn borer).

b. Specific Objective: Determine if physical and/or chemical differences exist between kenaf and roselle to explain differences in nematode resistance.

Progress: It was concluded that cluster crystals in scanning electron micrographs of nematode-infected kenaf and roselle roots were materials present naturally in the roots and merely concentrated by the nematode. One possible difference in kenaf and roselle root response to nematode infection is the formation of syncytial wall pits seen in a giant cell in roselle.

Larval hatch for egg masses located externally on tomato roots were determined after a series of infection times. These data evidence rhythmic fluctuations in hatch and allow prediction of optimum egg harvest times for culture maintenance and experimental use.

20160-006

Specific Objective: Study some environmental factors influencing the proportion of various alkaloids formed in Papaver somniferum plants.

Progress: Incising or lancing of immature poppy capsules of plants grown under controlled conditions increases the codeine content but lowers the morphine content of the entire above-ground plant. Codeine increases from 9.6 to 13.7 mg per plant; morphine declines from 19.9 to 16.5 mg per plant.

20160-007-A

Specific Objective: Broaden germ plasm base of Crambe abyssinica.

Progress: In an effort to broaden the germ plasm base of Crambe abyssinica, variety Meyer seed (with hulls) was treated with aqueous solutions of the chemical mutagen diethylsulfate (DES). Seed lots of 75 g each were treated at nine dosage levels, ranging from 0.5 to 8.0 ml DES/liter. Tests of each seed lot immediately after treatment showed pronounced decreases in germination with increasing dosage level. Seed from each dosage level (M1 seed) was planted in greenhouse beds and M_2 seed was harvested from these M_1 plants for each seed dosage level. Field plantings of M_1 (30-ft plots) and M_2 (headrows) seed were made in

April 1976. Matured plants were sampled from all M_1 plots and M_2 headrows. Primary evidence of induced variability is being sought from seed set data. Secondary evidence will be sought in oil analyses of seed from most M_2 headrows.

2. Progress Reports (Achievements):

20160-001

Publications:

POWELL, R. G. AND R. D. PLATTNER. Structure of a Secoisolariciresinol Diester from Salvia plebeia Seed. Phytochemistry 15 (1976):1963-1965.

SMITH, C. R., R. W. MILLER, D. WEISLEDER, W. K. ROHWEDDER, N. EICKMAN, AND J. CLARDY. Celorbicol, Isocelorbicol, and Their Esters: New Sesquiterpenoids from Celastrus orbiculatus. J. Org. Chem. 41 (1976):3264-3269.

MILLER, R. W., D. WEISLEDER, R. KLEIMAN, R. D. PLATTNER, AND C. R. SMITH. Oxygenated Fatty Acids of Isano Oil. Phytochemistry, in press.

MADRIGAL, R. V., G. F. SPENCER, R. D. PLATTNER, AND C. R. SMITH. Alkyl- and Alkenylresorcinols in Rapanea laetevirens Seed Lipids. Lipids, in press.

SMITH, C. R. Structural Analysis of Polyunsaturated Fatty Acids. Chapter in "Polyunsaturated Fatty Acids," AOCS Monograph, edited by R. T. Holman, in press.

MIKOLAJCZAK, K. L. Cyanolipids. Chapter in "Progress in the Chemistry of Fats and Other Lipids," edited by R. T. Holman, in press.

20160-002

Publications:

MIKOLAJCZAK, K. L., C. R. SMITH, AND D. WEISLEDER. Synthesis of Cephalotaxine Esters and Correlation of Their Structures with Antitumor Activity. J. Med. Chem. 20(3) (1976):328-332.

POWELL, R. G., C. R. SMITH, JR., AND R. V. MADRIGAL. Antitumor Activity of Sesbania vesicaria, S. punicea, and S. drummondii Seed Extracts. Planta Medica 30(1) (1976):1-8.

SMITH, C. R., R. G. POWELL, AND K. L. MIKOLAJCZAK. The Genus Cephalotaxus: Source of Homoharringtonine and Related Anticancer Alkaloids. Cancer Treat. Rep. 60 (1976):1157-1170.

SPENCER, G. F., R. D. PLATTNER, AND R. G. POWELL. Quantitative Gas Chromatography-Mass Spectrometry of Cephalotaxus Alkaloids. J. Chromatogr. 120 (1976):335-341.

20160-003

Publications:

KLEIMAN, R., M. B. BOHANNON, F. D. GUNSTONE, AND J. A. BARVE. Mass Spectra of Acetylenic Fatty Acid Methyl Esters and Derivatives. Lipids 11 (1976):599-603.

LIST, G. R. AND G. F. SPENCER. Fate of Jimsonweed Seed Alkaloids in Soybean Processing. J. Am. Oil Chem. Soc. 53 (1976):535-536.

SPENCER, G. F., R. D. PLATTNER, AND T. K. MIWA. Jojoba Oil Analysis by High-Pressure Liquid Chromatography and Gas Chromatography-Mass Spectrometry. J. Am. Oil Chem. Soc., in press.

SPENCER, G. F. Epoxyoctadecadienoic Acids from Crepis conyzaefolia Seed Oil. Phytochemistry, in press.

SPENCER, G. F., R. D. PLATTNER, AND R. W. MILLER. (-)-(S,S)-12-Hydroxy-13-Octadec-cis-9-enolide, A 14-Membered Lactone from Crepis conyzaefolia Seed Oil. Phytochemistry, in press.

SEYMOUR, F. R., M. E. SLODKI, R. D. PLATTNER, AND R. M. STODOLA. Methylation and Acetolysis of Extracellular Mannans from Yeast. Carbohydr. Res. 48 (1976):225-237.

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SESSA, D. J., H. W. GARDNER, R. KLEIMAN, AND D. WEISLEDER. Oxygenated Fatty Acid Constituents of Soybean Phosphatidylcholines. Lipids, in press.

SHOTWELL, O. L., M. L. GOULDEN, G. A. BENNETT, R. D. PLATTNER, AND C. W. HESSELTINE. Survey of 1975 Wheat and Soybeans for Aflatoxin, Zearalenone, and Ochratoxin. J. Assoc. Off. Anal. Chem., in press.

PRINCEN, L. H. Scanning Electron Microscopy in Coatings Research. Chapter 7 in "Treatise on Coatings," edited by Myers and Long, Vol. II (2), 377-411 (1976).

Other Reports:

PRINCEN, L. H. A New Look at Agriculture for Coatings Raw Materials. Abstract of presentation before 6th Annual Chicago Coatings Symposium, Symco '76.

PRINCEN, L. H. Potential Wealth in New Crops: Research and Development. Abstract of presentation before Society of Economic Botany, Champaign-Urbana, Illinois, June 1976.

PRINCEN, L. H. New Lipid Sources: Search and Development. Abstract of presentation before 10th IUPAC Symposium on Natural Products, Dunedin, New Zealand, August 1976.

PRINCEN, L. H. Chemical Riches in the Plant World. Abstract of presentation at Stauffer Chemical Company, Richmond, California, September 1976.

20160-005

Publications:

DELFEL, N. E. Ultraviolet and Infrared Analysis of Rotenone: Effect of Other Rotenoids. J. Assoc. Off. Anal. Chem. 59 (1976):703-707.

20160-006

Publications:

GROVE, M. D., G. F. SPENCER, M. V. WAKEMAN, AND H. L. TOOKEY. Morphine and Codeine in Poppy Seeds. J. Agric. Food Chem. 24(4) (1976):896-897.

TOOKEY, H. L., G. F. SPENCER, M. D. GROVE, AND W. F. KWOLEK. Codeine and Morphine in Papaver sonniferum L. Grown in a Controlled Environment. Planta Medica 30(4) (1976):340-348.

3. Plan of Work:

20160-001

Specific Objective: Isolate and characterize novel constituents of plant seeds with particular attention to those from plants identified as having potential pesticidal value.

Plan of Work: Unknown components will be isolated by various forms of chromatography or countercurrent distribution. Chemical derivatization, infrared, ultraviolet, nuclear magnetic resonance (NMR), and mass spectrometry will aid in identifying these constituents.

20160-002

a. Specific Objective: Develop synthetic methods that will facilitate conversion of cephalotaxine to harringtonine, homoharringtonine, or other esters with antitumor activity.

Plan of Work: Improved methods will be sought for preparation of the α-keto-ester precursor of harringtonine with appropriate blocking groups. New approaches for attaching the carbomethoxymethyl side chain will be tested.

b. Specific Objective: Preparation of new plant extracts.

Plan of Work: New extracts will be submitted as rapidly as available facilities and personnel allow. Reconfirmation samples will be prepared as requested by laboratories that perform bioassays.

c. Specific Objective: Fractionation of "confirmed actives."

Plan of Work: Isolation and characterization of active compounds in Sesbania drummondii and Cephalotaxus mannii will be pushed as far as possible. As many of the other confirmed actives will be fractionated as time and material permit.

20160-003

a. Specific Objective: Discover and characterize novel plant seed oils.

Plan of Work: Seed, collected in the wild, will be analyzed for oil and protein. The seed oil will be subjected to gas chromatography (GC), thin-layer chromatography (TLC), IR, and UV spectrometry to detect unusual composition. Selected oils will be converted to esters and again analyzed by GC and TLC. Unknown components will be characterized by GC-MS, IR, NMR, and other analytical techniques.

b. Specific Objective: Provide compositional data in support of plant breeding and agronomic research.

Plan of Work: Samples, provided by plant breeders, will be analyzed for oil, protein, and fatty acid composition. In selected samples, amino acid analysis will be included. Emphasis will be given to the Brassica Improvement Program, new crambe germ plasm, Stokes' aster, and okra.

20160-004-A

Specific Objective: To develop seeds of Brassica cultivars with low glucosinolate content and high erucic acid content.

Plan of Work: Development of a high erucic acid and low glucosinolate content in seed of the genus Brassica will continue.

20160-005

a. Specific Objective: Compare sterol-steroid levels in kenaf and roselle for possible correlation with nematode resistance.

Plan of Work: Fractionate and chemically analyze sterols and steroids in Hibiscus roots on hand.

b. Specific Objective: Discover and characterize useful pesticidal activity in plant seeds.

Plan of Work: Continue extraction and fractionation of seeds from NRRC collection and test in-house as well as through collaborators for activity against major pests.

20160-006

Specific Objective: Continue study of environmental factors influencing the proportion of various alkaloids formed in Papaver plants.

Plan of Work: Vary selected environmental factors that may influence the production of desirable alkaloids.

20160-007-A

Specific Objective: Complete data collection on currently available M_1 and M_2 seeds. Evaluate data to date and progress towards broadening crambe's germplasm base.

Plan of Work: Data collection on seed set and oil content of seeds will be completed. Statistical analyses of the data will determine the effectiveness of different DES dosage levels in mutagenically inducing genetic variability in vigorous plants. If effective dosage levels are found, then a larger population of plants can be screened for morphological and biochemical mutants of agronomic value.

B. Summary of Progress for Extramural Projects:

1. Progress Report (Narrative):

8002-20160-228

Sixteen species of Umbelliferae are being investigated for their bacteriostatic/bacteriocidal properties. Essential oils from seed from seven additional species have been extensively surveyed for their chemical composition. These species include: Heracleum candicans (26% bornyl acetate, 12% amyl acetate), Pimpinella acuminata (70% n-methyl conine), Pimpinella anisum (84% anethole), Ferula assafoetida (35% secondary butyl propenyl disulphide, 21% myristic acid), Scandix pectin-veneris (38% lauric acid, 11% 2-undecanone, 20% coumarins), Petroselinum crispum (65% myristicin), and Ligusticum elatum (18% hydrocarbons, 21% coumarins).

8001-20160-236

An additional 350 plants have been collected and identified. About 175 seed samples have been collected with about 100 having been screened. Unusual components have been detected in several oils. Analyses by chromatography have led to the discovery of several species with oleic-linoleic rich oils

which deserve agronomic evaluation. Cyanolipids were discovered in a member of the Boraginaceae although verification of the plant identification is still forthcoming. Other characterization work to identify unusual constituents has continued. In this regard, a previously unknown fatty acid (9,14-dihydroxy-octadecanoic) was isolated from Peganum harmala seed oil.

8001-20160-244:

The fatty acid composition of the total seed lipids of Coriandrum sativum has been determined as a function of maturity. An unidentified fraction appears to consist of free aldehydes, which may be essential oil constituents. The triglyceride fraction has been characterized, and the phospholipid fraction is being analyzed. Momordica charantia seed oil was also analyzed as a function of seed maturity. Linolenic acid decreased from 19.7% in the oil of 10-day-old seed to only a trace in the oil of 22-day-old seed. Conjugated trienoic acid was absent at 10 days of age, but increased to 56.9% at 22 days. The phospholipids did not contain any conjugation at any age.

8001-20160-245

Tissues from a broad range of plant species have been cultured on Murashige and Skoog medium (modified by increasing inositol level to 2 or 5 g/liter and by adding a complete vitamin supplement) and have been analyzed for secondary metabolites: Papaver somniferum and P. rhoeas (5.6% and 1.3%, total morphane alkaloids, resp.), Atropa belladonna, Datura metel, Hyoscyamus niger, and Solanum nigrum (0.5%, 0.2%, 0.7%, and 1.5% total tropane alkaloids, resp.; S. nitrum also contained 0.004% solasodine). S. elaegnifolium, S. khasianum, and S. aviculare (0.03%, 0.005%, and 0.09% solasodine, resp.; the latter also produced 0.8% diosgenin.) Cholesterol added to the medium (90 mg/liter) stimulated solasodine synthesis in S. aviculare, ascorbic acid (1 g/liter) increased pyrethrins in T. erecta and morphane alkaloids in P. somniferum. Tyrosine (0.5 g/liter) also reportedly increased total morphane alkaloid concentration in P. somniferum.

2. Progress Reports (Achievements):

8001-20160-236

AHMAD, M. U., S. K. HUSSAIN, M. AHMAD, AND S. M. OSMAN. Cyclopropenoid Fatty Acids in Sida acuta and S. rhombofolia Seed Oils. J. Am. Oil Chem. Soc., in press.

HUSSAIN, S. K., SORITA SINHU, A. A. ANSARI, M. AHMAD, AND S. M. OSMAN. Studies on Herbaceous Seed Oils. I. J. Oil Technol. Assoc. India, in press.

8001-20160-245

KHANNA, P. AND R. KHANNA. Production of Major Alkaloids from in vitro Tissue Cultures of Papaver somniferum Linn. Indian J. Exp. Biol. 14 (1976):628-630.

KHANNA, P. AND R. KHANNA. Endogenous Free Ascorbic Acid and Effect of Exogenous Ascorbic Acid on Growth and Production of Pyrethrins from in vitro Tissue Culture of Tagetes erecta L. Indian J. Exp. Biol. 14 (1976):630-631.

KHANNA, P. AND R. KHANNA. Effect of Various Auxins on Growth and Production of Tropane Alkaloids from in vitro Tissue Culture of Datura metel Linn. Indian J. Pharm. 38 (1976):120-123.

PHYSIOLOGICAL AND BIOCHEMICAL TECHNOLOGY TO IMPROVE CROP PRODUCTION

A_1 Technological Objective: Improve biological conversion of solar energy for increased crop production by increasing the efficiency of photosynthesis, translocation, and associated metabolism.

1. Progress Report (Narrative):

20170-001

a. Specific Objective: Measure the action spectrum of Chlorella pyrenoidosa, Nitzschia closterium, and of other algal species to assess the efficiency of specific carotenoids and other chloroplast pigments in sensitizing photosynthesis. Armed with the knowledge of which pigments are photosynthetically active, the plant breeder can hope to modify crop plants for higher light absorption and efficiency of use.

Progress: Plans to measure action spectra particularly in the region of carotenoid absorption (photosystem II) have been implemented by setting up two dye tunable lasers (pumped by a single Argon laser). Required light mixing optical systems have been constructed. Equipment for measuring oxygen, fluorescence, and radiant energy absorbed have been acquired and/or adapted. Preliminary measurements of quantum yield have been made. A team of scientists has now been assembled with disciplines ranging from physics and chemistry through biology. It includes a plant physiologist, biochemists, chemists, a physical chemist, and a physicist (biophysics).

A controlled-environment growth facility for higher plants and equipment for batch and continuous culture for unicellular algae are in use. Required physical equipment such as dye tunable lasers, a mass spectrometer, spectrofluorimeters, oxygen electrodes, and radiometers have been acquired and are being interfaced for on-line computer acquisition of data.

b. Specific Objective: In cooperation with the U.S. Regional Soybean Laboratory, Urbana, study photorespiration in the presence of photosynthesis in order to reduce loss of efficiency caused by photorespiration.

Progress: As planned, the methodological phase of setting up the mass spectrometer equipment for measuring $^{18}O_2$, $^{16}O_2$, $^{12}O_2$, $^{13}O_2$ exchanges in algae and in higher plants has proceeded during the planned absence from Urbana of the cooperating scientist on the photorespiration problem.

c. Specific Objective: Extend measurement of action spectra to higher plants with high carotenoid contents such as yellow isolines of soybeans, commercial cultivars of yellow peanuts, and pale yellow tobacco.

Progress: Yellow isolines of soybeans from Clark and Harosoy have been supplied by Dr. R. L. Bernard, ARS plant geneticist at Urbana, and are being grown and being analyzed for carotenoid pigment composition of leaves and for resistance to UVB radiation in accordance with last year's plans.

A high-performance liquid chromatograph (HPLC) designed specifically for chloroplast pigment separations and quantitative analyses has been constructed and has been adapted for computer monitoring and calculations. HPLC has been used for analyses of pigments in species of higher plants and of algal families. This HPLC procedure of pigment separation and quantitative analysis constitutes a major methodological advance and should replace all other procedures for quantitative analysis of pigments.

d. Specific Objective: To grow and maintain certain algae strains for studies on photosynthesis in fermentation.

Progress: Strains of the following algae were tested for growth and maintenance in algae media known as IAM, Erd, PMM, SCHR, CC, and F/2: IU-161 Porphyridium cruentum; LB-200 Duneliella sp.; IU-642 Nitzschia closterium; IU-646 Nitzschia closterium; Em #3 Chlorella sp.; and Cyanidium caldarium. The IAM medium was also supplemented with a biotin (1.0 µg/L), thiamin·HCl (0.2 mg/L), cyanocobalamin (1.0 µg/L) vitamin mixture. The following media supported adequate growth for maintenance of the strains: IAM-1 (a medium for sea diatoms supplemented with the vitamin mixture) and Erd. (Erdschreiber solution) for IU-161 Porphyridium cruentum; S-2SW (soil extract-2 X sea water solution) or S-4SW for LB-200 Duniella sp.; IAM-1 for strains IU-642 and IU-646 of Nitzschia closterium; Yxt pH 7 (yeast extract malt extract glucose agar adjusted to pH 7) for Em #3 Chlorella sp.; and CC (Cambridge CC medium for blue-green algae adjusted to pH 4.5) for the strain of Cyanidium caldarium. Therefore, the above strains of algae can be readily maintained with periodic transfer in test tubes containing certain liquid or agar media when incubated under light-dark conditions.

2. Progress Reports (Achievements):

20170-001

Publications:

ESKINS, K., C. R. SCHOLFIELD, AND H. J. DUTTON. High Performance Liquid Chromatography of Plant Pigments. J. Chromatogr. Accepted for publication.

3. Plan of Work:

20170-001

a. Specific Objective: Identify specific carotenoid pigment sensitizers of photosynthesis by quantum efficiency measurements on species from algal families and on higher plants in order to advise plant breeders of how to modify crop plants for higher light absorption and efficiency.

Plan of Work: Construct system for irradiating algae such as species Chlorophyceae and Bacillariophyceae, which differ in carotenoid composition, and higher plants such as yellow isolines of soybeans, commercial cultivars such as yellow peanuts and pale yellow tobacco. With monochromatic light from two dye tunable lasers, measure absorbed light quanta and yield of oxygen and fluorescence and thus the action spectrum of carotenoids composing Photosystem II.

b. Specific Objective: Study photorespiration in the presence of photosynthesis in order to reduce loss of efficiency caused by photorespiration in cooperation with ARS scientists stationed at Urbana, Illinois.

Plan of Work: Laser and mass spectrometric equipment for irradiating and for monitoring $^{18}O_2$, $^{16}O_2$, $^{12}CO_2$, and $^{13}CO_2$ exchanges in algae and higher plants will be assembled in order to assess the magnitude and the influence of environmental parameters on photorespiration, thus contributing to NRRC's part of the cooperative research.

c. Specific Objective: Initiate studies on the mechanism and efficiency of energy transfer from carotenoid to chlorophyll molecules in carotenoid-chlorophyll-protein systems isolated from plant sources and those synthesized from individual purified pigment components.

Plan of Work: Carotenoproteins will be isolated from various higher plant and algal sources by detergents, sonications, precipitations, chromatography, etc. Synthetic systems will be devised from pure isolated plastid pigments in emulsion systems, or alternatively, using monolayer techniques to deposit films of chlorophyll and carotenoid pigments with specific spacings and/or orientations.

Light energy transfer from carotenoid to chlorophyll molecules will be determined in isolated and synthetic systems by measuring fluorescence excitation spectra. Results on these synthetic isolated systems at the in vitro level will be used to interpret in vivo phenomena observed in chloroplasts of living plant cells.

A_2 Technological Objective: Improve nitrogen-fixation efficiency of bacteria-plant associations and develop nitrogen-fixing capabilities in crops lacking this capability in order to reduce energy requirements for crop production.

1. Progress Report:

20170-002

a. Specific Objective: Isolate and characterize microorganisms associated with the Azolla-blue-green algae symbiosis.

Progress: The blue-green algae in Azolla have now been isolated. This is the first time these organisms have been isolated and purified for study. The two bacteria associated with the symbiosis have also been isolated and identified as Caulobacter and Alcaligenes, respectively.

b. Specific Objective: Study nitrogen fixation and hydrogen evolution in blue-green algae and algal preparations derived from Azolla plants.

Progress: Conditions for optimum nitrogen fixation and hydrogen evolution have been determined in laboratory and continuous fermenter studies.

c. Specific Objective: Perform biochemical studies of nitrogen transfer in intact symbiotic system.

Progress: Isotope studies with N^{15} and quantitative analyses of nitrogenous pools of Azolla have been completed. Plants were found to assimilate N_2, NH_4, and NO_3 at similar rates and accumulates large amounts of NH_3 intracellularly during N_2 fixation.

d. Specific Objective: Transfer of nitrogen-fixing genes from Rhizobium and Agrobacterium.

Progress: A survey of Rhizobium strains for their ability to fix nitrogen in vitro has been completed. From examination of several hundred strains, 70 have been shown capable of fixation and have been selected for further study.

2. Progress Reports (Achievements):

20170-002

Publications:

NEWTON, J. W. Photoproduction of Molecular Hydrogen by a Plant-Algal Symbiotic System. Science 191 (1976):559-561.

NEWTON, J. W. AND J. F. CAVINS. Altered Nitrogenous Pools Induced by Azolla-Anabaena Azolla Symbiosis. Plant Physiol. 58 (1976):798-799.

STRANDBERG, G. W. Continuous Photoproduction of Molecular Hydrogen by Anabaena floss-aquae. Dev. Ind. Microbiol. 17 (1976):

Other Reports:

NEWTON, J. W. Production of Molecular Hydrogen by a Plant Algal Symbiotic System. Presented at Brookhaven National Laboratory, Upton, Long Island, New York, April 8, 1976.

NEWTON, J. W. Energy Inputs into Agriculture. Presented at Sigma Xi, Peoria, Illinois, September 21, 1976.

NEWTON, J. W. Studies on a Fern Algal Symbiosis. Presented at University of California, San Diego, California, November 23, 1976.

Fertilizer and Fuel from Plants. Agric. Res. 25 (1976):7-9.

Alga-Fern System Releases Hydrogen from Water. BioScience 26 (1976):652.

Plants Produce Elements of Fuel and Fertilizer. Wisconsin Agriculturalist, October 9, 1976:A-58.

Plants Produce Fuel and Fertilizer. Des Moines Register, August 22, 1976.

STRANDBERG, G. W. Continuous Photoproduction of Molecular Hydrogen by Anabaena floss-aquae. Presented at Society for Industrial Microbiology Meeting, Jekyll Island, Georgia, August 15-20, 1976.

3. Plan of Work:

20170-002

a. Specific Objective: Study the physiology of the individual partners of the plant-algal symbiosis and attempt to recombine them into an effective nitrogen-fixing plant.

Plan of Work: Blue-green algae isolated from Azolla will be examined and the properties of nitrogen fixation in these strains will be compared with those of free-living blue-green algae. Possible roles played by other microbial members of the symbiosis will be studied, especially with regard to their hydrogen metabolism. Genetic studies of these organisms will be initiated and conditions of UV killing and mutagenesis studied.

b. Specific Objective: Develop genetic transfer system for introduction of nitrogen fixation into Agrobacterium strains, and study infection of carrot tissue.

Plan of Work: Invasiveness and tumorigenicity, growth, and survival of Agrobacterium auxotrophs will be studied using carrot and root cultures. Complementary Rhizobium auxotrophs will be prepared and used to transfer nif genes. Reversed transfer of invasiveness to Rhizobium will also be attempted.

A_3 Technological Objective: Develop new and improved cell and tissue culture technology for plant improvement through increased genetic diversity and rapid vegetative propagation.

1. Progress Reports (Narrative):

20170-003

a. Specific Objective: Increase knowledge of biosynthesis alkaloids in Cephalotaxus harringtonia.

Progress: Cephalotaxine levels did not change with age in a small laboratory-grown plant while antitumor cephalotaxine esters increased fivefold, accounting for more then 98% of the total cephalotaxine in the extracts. Levels in leaves were 2 to 4 times those in stems and tended to increase with tissue age while stem levels declined.

b. Specific Objective: Produce sufficient callus tissues of C. harringtonia for analysis.

Progress: The presence of cephalotaxine and all of its known antitumor esters were confirmed in cultures by GC-MS. In addition, a new alkaloid ester, named homodeoxyharringtonine, was discovered which should have high antitumor activity by analogy with known compounds. Both homodeoxyharringtonine and deoxyharringtonine were found only in the culture medium; all other C. harringtonia alkaloids were found both in the callus and medium in low concentrations. In cultured tissues, unlike the parent plant, cephalotaxine comprised 40 to 60% of the total alkaloids.

c. Specific Objective: Grow C. harringtonine cells in liquid suspension.

Progress: C. harringtonia cells were adapted to liquid suspension culture by reducing the microelement concentration of Murashige and Skoog's medium to one-fourth, normal levels being toxic. Cells have been successfully maintained through several transfers and will be compared to callus tissues in further experiments.

d. Specific Objective: Establish suitable growing conditions for Tephrosia vogelii callus cultures.

Progress: T. vogelii seedlings were successfully callused on modified Murashige and Skoog medium supplemented with 10 mg/1 NAA and 15% (v/v) coconut milk. Optimum conditions were found to be 10 or 20 mg/1 NAA and 4 mg/1 kinetin which gave a fivefold increase in growth rate compared to the initial medium.

2. Progress Reports (Achievements):

20170-003

Publication:

DELFEL, N. E. AND J. A. ROTHFUS. Antitumor Alkaloids in Callus Cultures of Cephalotaxus harringtonia. Phytochemistry, in press.

3. Plan of Work:

20170-003

a. Specific Objective: Study post-harvest changes in the concentration, composition, and distribution of Cephalotaxus harringtonia antitumor alkaloids.

Plan of Work: Harvest C. harringtonia plants, or portions thereof, and store for varying periods under different conditions of humidity, etc. Analyze extracts for alkaloids by GLC and GC-MS.

b. Specific Objective: Study alkaloid production by C. harringtonia cells in liquid suspension culture.

Plan of Work: Grow sufficient amounts of cells on rotary shakers to permit identification and quantitation of antitumor alkaloids produced and compare to results with callus tissues. Follow alkaloid production as a function of time.

c. Specific Objective: Determine which microelement in standard Murashige and Skoog medium is responsible for toxicity to C. harringtonia cells.

Plan of Work: Test effect of each of the eight elements in M/X-microelement mixture on growth rate of C. harringtonia cells.

d. Specific Objective: Devise means of growing single cells of C. harringtonia in culture to permit study of clones.

Plan of Work: Attempt to grow single cells via agar-gel plating, nurse culture, micro-culture, or innovative techniques.

e. Specific Objective: Determine whether rotenoids are produced by Tephrosia vogelii in callus culture.

Plan of Work: Characterize by GC-MS and quantitate by GLC the rotenoids present at various stages in the establishment and growth of callus cultures: seed germination, callus induction, and callus propagation.

A_4 Technological Objective: Develop technology for reducing damage to crop plants from air pollution.

1. Progress Report (Narrative):

20170-001

Specific Objective: Study the effect of ultraviolet light and ozone on the photosynthetic and respiratory rates of algae, higher plants, and yellow variants in relation to the problem of depletion of ozone in the upper atmosphere.

Progress: Study of the effects of UV-B on the photosynthesis and respiration of Chlorella and on the isolines of Clark and Harosoy soybeans were initiated during the past summer with the aid of two NSF Awardees coming from Knox College. Equipment funds supplied by ERDA were used to implement work on this technological objective in FY 1976. A significant methodological advance was made in the generation of simulated UV-B with a simple, practical, and widely available device. It was discovered that the fluorescent lamp coating #RUL-3000 gives its major emission between 260 and 320 nm which is quite precisely the region specified as UV-B. However, the lamp also emits exciting lines of mercury including the bacteriocidal resonance radiation line at 254 nm. The simple expedient of fitting a Corex D glass tube over the fluorescent tube suffices to remove this lethal radiation. Although use of ultraviolet transmitting purple glass filters will reduce visible light and still pass UV-B, in most experiments the comparatively weak visible light added to normal levels of visible light have no measurable effect. This discovery of a simple source has been made available to

scientists at this Center working on UV-B effects on nitrogen fixation, photosynthesis, and respiration and to those at the ARS Agricultural Research Center, Beltsville, Maryland, who have corroborated our observations with spectroradiometric measurements.

3. Plan of Work:

20170-001

Specific Objective: Confirm and expand observations on the effect of ultraviolet light and ozone on the photosynthetic and respiratory rates of algae, and of higher plants, and yellow variants in relation to the problem of depletion of ozone in the upper atmosphere.

Plan of Work: As a research approach, respiration of Chlorella pyrenoidosa (green algae) and Pheodactylum tricornatum (yellow-brown algae) will be followed in darkness and the effect of measured dosages of UV-B on oxygen uptake will be studied. In similar experiments on higher plants, the effects of measured dosages of ultraviolet light upon the combined photosynthetic and respiratory mechanisms will be monitored and interpreted in part with respect to protective carotenoid pigmentation.

BIOLOGICAL AGENTS FOR PEST CONTROL

A. Technological Objective: New and improved technology for increase and conservation of introduced and native biological agents for control of insects, weeds, plant pathogens, and other pests.

1. Progress Report (Narrative):

20260-001

a. Specific Objective: Investigate biochemistry and toxicity of insecticidal products of Bacillus thuringiensis, including the β-exotoxin and delta-endotoxin, in order to improve understanding of their mode of action and so facilitate their use in the field.

Progress: The inhibition of bacterial ribonucleic acid polymerase (RNAp) by β-exotoxin was more thoroughly investigated. RNAp isolated from vegetative cells of Escherichia coli and B. thuringiensis are more sensitive to exotoxin than stationary-phase RNAp. Also, exotoxin inhibition of E. coli core polymerase was 15-17 percent less than corresponding inhibition of holoenzyme. These data suggest a possible correlation between diminishing exotoxin sensitivity and loss of sigma factor from the core polymerase complex during sporulation.

A new process for the detection of β-exotoxin in cell-free extracts involving high-pressure liquid column chromatography (HPLC) is being evaluated. A single peak emerges from the column at approximately 2.35 minutes after sample injection and is identifiable as exotoxin. However, more work is required to relate the identity of the HPLC peak with the toxic activity towards the common house fly as used in the normal exotoxin assay.

The investigation of deltaendotoxin and its toxic subunits has advanced to the stage where Renographin-purified paraspores have been enzyme digested and are to be examined by electrophoresis for major protein species. Several B. thuringiensis strains are included in the initial survey, including representatives of serotypes I, III, and VII. Purification of crystals requires at least three density gradients centrifugations in Renographin, resulting in approximately 99-percent pure crystals.

b. Specific Objective: Attempt to improve the preparation of B. thuringiensis in encapsulated form for use against larvae of the Japanese beetle and other pest insects.

Progress: Encapsulation of B. thuringiensis spores and crystals in wood rosin achieves protection from environmental influences and the rosin dissolves at pH 9 to release viable spores and active crystals. The encapsulated product appeared to be inactive against small lepidoptera (cabbage looper) during initial studies, but field tests performed against Japanese beetle larvae (a coleopteran species) was moderately successful. Damage caused by the B. thuringiensis crystal to the mid-gut lining (epithelial cells) of Japanese beetle larvae was shown by photomicrographs of thin sections to be as severe and extensive as occurs in susceptible lepidoptera. Damage to mid-gut of Japanese beetle larvae by B. thuringiensis crystalline protein leads to bacteriemia and death. The mid-gut contents of Japanese beetle larvae are not always at pH 9.5 as reported by other researchers. When actively feeding, the larval midgut pH is from 9.5 to 10.2 and such larvae are susceptible to the crystalline endotoxin from B. thuringiensis. When the larvae cease feeding as in diapause, gut pH drops to 6.5 to 8.5, consequently, these larvae are usually not susceptible to B. thuringiensis.

The news of a resurgence of Japanese beetle infestations in Connecticut has renewed dialogue and cooperation between NRRC and scientists at the ARS Japanese Beetle Laboratory located at Wooster, Ohio. The rising occurrence of infestations are due apparently to a diminished effectiveness of milky disease. A new variant strain of B. popilliae, the causative agent of milky disease, has been isolated from diseased Connecticut Japanese beetle larvae. The variant is characterized by: (1) Abnormally large paraspores and multiple paraspores per endospore, (2) depressed sporogenesis resulting in diminished spore yields per diseased larva, and (3) appears to be infective in laboratory injection tests.

A vegetative culture of NZ-1, a Bacillus that causes milky disease of a grass grub in New Zealand and strongly resembles B. popilliae, has been obtained for the first time. The NZ-1 Bacillus also has an abnormally large, loosely attached paraspore and is not very infective to the grub. Our vegetative culture is being studied by researchers in New Zealand.

c. Specific Objective: Determine infectivity of various Entomophthora spores (and other fungi) to the larvae of the Japanese beetle, and examine the influence of these spores on the dose requirements of B. popilliae for milky disease production in the larvae.

Progress: In the previous year, research emphasis was placed on the pathogenicity of various fungi known to be associated with several insect pests. Specifically, to determine the lethal effect of fungi (as secondary invaders) on milky diseased Popilliae japonica third instar larvae. Data from these experiments indicated that concentrations of about 1×10^9 fungal spores per gram of soil is needed in feeding experiments to accelerate the death of P. japonica larvae previously field infected with B. popilliae. The milky diseased larvae are highly susceptible to the fungi Nomuraea rileyi, Metarrhizium anisophiae, and Beauveria bassiana. In order to continue the search for fungi and bacteria that may be pathogenic to Coleoptera, we proposed (in our last report) to accumulate large stocks of entomogenous microorganisms. Since our last report, we have acquired gram quantities of these microorganisms to be tested for infectivity against P. japonica larvae.

A general medium for growth and sporulation of entomogenous fungi was developed and used for large-scale fungal production. The medium consists of yeast extract, 0.4 percent; soluble starch, 1.5 percent; dibasic potassium phosphate, 0.1 percent; tryptose, 0.1 percent; magnesium sulphate, 0.05 percent; and agar, 2.0 percent. A minimum of 4×10^{12} fungal conidia, per each strain of fungi grown, were collected, dried, and stored for future infectivity studies. The fungi harvested were Nomuraea rileyi, Metarrhizium anisopliae, Beauveria bassiana, Entomophthora apiculata, E. coronata, E. destruens, and E. thaxteriana. Using known bacterial sporulation media, similar quantities of spores from B. thuringiensis, B. subtilis, B. alvei, B. cereus, B. apiarius, and B. megaterium were also collected for future studies.

Initial efforts to obtain all "typed" cultures of fungi belonging to the Order Entomophthorales have been completed. This achievement involves an agreement by Drs. Richard S. Soper and Richard A. Humber of the N. E. Plant, Soil, and Water Laboratory, University of Maine, at Orono, and Dr. J. P. Latge, Pasteur Institute, to obtain (worldwide) cultures of Entomophthora and deposit them in the ARS Culture Collection for future insect pathogenicity studies.

2. Progress Reports (Achievements):

20260-001

Publications:

JOHNSON, D. E. Bacterial Membrane Transport of β-Exotoxin, An Anti-Metabolite of RNA Synthesis. Nature 260 (1976):333-335.

SHARPE, E. S. Toxicity of the Parasporal Crystal of Bacillus thuringiensis to Japanese Beetle Larvae. J. Invertebr. Pathol. 27 (1976):421-422.

SHARPE, E. S. Toxicity of the Bacillus thuringiensis Paraspore to Japanese Beetle Larvae: Susceptibility, Mid-Gut pH, and Some Histopathological Effects. Proc. IX Ann. Meeting, Soc. Invertebr. Pathol., Kingston, Ontario, Canada, 1976. Published February 1977.

Other Reports:

JOHNSON, D. E. Inhibition of RNA Synthesis by β-Exotoxin and Its Potential Use as an Insecticide. Presented at Northern Regional Research Center, Peoria, Illinois, May 17, 1976.

JOHNSON, D. E. Bacterial RNA Polymerase Inhibition Kinetics Caused by β-Exotoxin. Presented at Insect Pathology Research Institute, Sault Ste. Marie, Ontario, Canada, February 15, 1977.

SHARPE, E. S. Toxicity of the Bacillus thuringiensis Paraspore to Japanese Beetle Larvae: Susceptibility, Mid-Gut pH, and Some Histopathological Effects. Presented at IX Ann. Meeting, Soc. Invertebr. Pathol., Queens University, Kingston, Ontario, Canada, August 29-September 2, 1976.

ST. JULIAN, G. Agricultural Research Service Biological Research Against Pest Insects: 1920-1976. Presented at Webster School, Peoria, Illinois, January 6, 1977.

ST. JULIAN, G. Recent Advances in Popillia japonica Research. Presented at Eastern Illinois University, Charleston, Illinois, February 7, 1977.

3. Plan of Work:

20260-001

a. Specific Objective: Investigate subunit chemistry and toxicity of the delta-endotoxin from B. thuringiensis, and continue attempts to devise a detection and assay method for β-exotoxin, another toxin produced by B. thuringiensis.

Plan of Work: The recent acquisition of techniques and materials necessary for the culturing and maintenance of insect cell lines will provide a means for performing bioassays on toxic substances. Fractions resulting from enzyme-digested endotoxin preparations will be analyzed for toxicity, as well as examined electrophoretically. The toxic response between β-exotoxin and insect tissue will also be examined.

b. Specific Objective: Accomplish microencapsulation of B. thuringiensis spores and crystals or dissolved and digested crystal product for use against Japanese beetle larvae, resistant lepidoptera pests, and other insects not susceptible to the intact crystal.

Plan of Work: Investigate suitability and protective ability of man-made polymers as well as different formulations of wood rosin in modified encapsulation procedures.

c. Specific Objective: Determine if toxic B. thuringiensis crystals can be used to facilitate invasion of Japanese beetle hemocoel by vegetative cells of B. popilliae or other pathogenic microorganisms for insects such as S. marscescens or Entomophthora spores.

Plan of Work: Hand feed various concentrations of B. thuringiensis crystal and viable vegetative cells of B. popilliae in combination to infect Japanese beetle larvae with milky disease. Also feed combinations of B. thuringiensis crystals and other entomopathogens. If laboratory trials produce positive results, encapsulate effective combinations and attempt field control.

d. Specific Objective: Establish chemical basis for diminished infectivity of the new variant strain of B. popilliae isolated from diseased Japanese beetle larvae from Connecticut.

Plan of Work: Produce quantity of variant spores in artificially infected Japanese beetle larvae or Cyclosephala larvae. Develop technique to remove abnormally large paraspores and compare electrophoretic pattern with that of normal paraspores of B. popilliae.

e. Specific Objective: Continue screening for fungi and bacteria that may be pathogenic or toxic to Popillia japonica.

Plan of Work: Entomopathogenic microorganisms shall be cultivated on appropriate solid media to the point of conidiation; healthy P. japonica larvae will be allowed to feed on conidia or vegetative tissue for various time periods, removed, and incubated in a soil environment. The biological effect of entomopathogens will then be evaluated. Data will be used to determine the most promising microorganisms for further research.

f. Specific Objective: Determine the specific entomopathogen dose (number of conidia or spores) required to effect 100-percent kill of P. japonica larvae.

Plan of Work: Species of Nomuraea, Beauveria, and Entomophthora spores shall be mixed with soil at various numbers per gram of soil. Larvae then shall be fed the spore-containing soil to determine the most effective dose for kill.

g. Specific Objective: Cooperate with the ARS Biological Control of Insects Research Unit (BCIR), Columbia, Missouri, in screening for potentially toxic materials produced by various fungi and bacteria against neonatal cabbage looper larva.

Plan of Work: Develop and employ efficient methods for supplying large (gram to as much as kilogram) quantities of fungal and bacterial preparations, including whole-culture filtrates, spent growth media, cellular aliquots, and selected semipurified components (conidia, etc.). These preparations, usually in dessicated form, will be sent to BCIR for bioassay.

h. Specific Objective: Investigate the complete morphological development of Nomuraea rileyi, an effective pathogen of insects damaging to soybean plants.

Plan of Work: Study the development of this entomopathogen on specific media at various environmental conditions using microscopy. Develop methods for studying the potential dimorphic nature of this fungus.

AGRICULTURAL CHEMICALS TECHNOLOGY FOR CROPS PROTECTION AND MODIFICATION

A. Technological Objective: Develop new information on natural bioconstituents and related synthetic compounds that control physiological and biochemical processes for the development of chemicals to modify plant structure and processes.

1. Progress Report (Narrative):

20290-001

a. Specific Objective: Utilize the developed procedure for fractionation of rape pollen extracts containing brassin plant growth promoting agents to provide adequate amounts of compounds for characterization.

Progress: Guided by bioassays conducted at the ARS Agricultural Research Center, Beltsville, Maryland, extracts of rape pollen were fractionated by two-phase liquid-liquid partitioning followed by adsorption chromatographies. Active and inactive fractions, both consisting mainly of mixtures of glucose esters of fatty acids, were obtained. The active material, which was closely associated with these esters, was separated from them by reverse-phase high-performance liquid chromatography to afford minute amounts of brassins. This chromatographic technique also resolves glucose ester mixtures.

b. Specific Objective: Identify isolated compounds.

Progress: The glucose esters were identified as 6-glucosyl palmitate, linolenate, linoleate, and oleate. These esters are all inactive as plant growth promoters. Nicotinamide was characterized in an active fraction but is also inactive.

2. Progress Reports (Achievements):

20290-001

Publications:

None

Other Reports:

MANDAVA, N., J. F. WORLEY, J. D. WARTHEN, JR., M. D. GROVE, AND P. E. PFEFFER. Progress Report on Isolation and Identification of Brassins. Presented by N. Mandava at Ninth International Conference on Plant Growth Substances, Lausanne, Switzerland, August 30-September 4, 1976.

3. Plan of Work:

20290-001

Specific Objective: Isolate adequate quantities of pure brassin plant growth promoters for characterization.

Plan of Work: Reverse-phase chromatographic techniques will be developed and incorporated into the current fractionation procedure to process additional rape pollen extracts.

STRUCTURES, EQUIPMENT, AND SYSTEMS FOR LIVESTOCK PRODUCTION

Summary of Progress for Extramural Projects:

Progress Report (Narrative):

3090-20401-001-C

A pilot plant for the anaerobic digestion of animal wastes was designed and built at U.S. Meat Animal Research Center (MARC), Clay Center, Nebraska, under contract with Hamilton Standard Division of United Technologies. The facility was designed to operate with varied digestible wastes over a range of operating conditions.

The primary purpose of the pilot plant is to provide operational data on digestion of cattle wastes and eventually other animal wastes and crop residues.

The four subsystems of the pilot plant are: slurry (100 pounds d.b./day fermentation (1250 gallon), solids recovery (centrifuge), and animal feed processing (drier, mill); full instrumentation is provided and appropriate analytical capability is available.

The pilot plant was tested by static load and by material flow prior to startup. On November 1, 1976, the digestion was started by introduction of raw cattle waste at low solids concentration and infrequent feed rate; no inoculum was used. Subsequently, feed rate was slowly increased and stable operation was obtained at 0.25 pounds/cu ft/day and operating temperature of 52°C. Gas production of 50 percent CH_4 is 8 cu ft/pound vs introduced or 14 cu ft/pound vs destroyed at retention times of 11 days. This initial rate is stable and will be used as a base for planned parametric tests at higher loading rates, temperature, and shortened retention time.

The contractor has completed their design, fabrication, procurement, construction, and startup obligations; a detailed operation manual has been supplied to ARS by the contractor. A second volume containing component specifications and vendor literature will be supplied shortly with the test and startup data.

Operation of the pilot plant has been turned over to ARS personnel at MARC.

CONTROL OF CATTLE DISEASES

A_1 Technological Objective: Improve methods to minimize losses from disease (fusarium toxins, mycotoxins, and other toxicity).

1. Progress Report (Narrative):

20420-001A

Specific Objective: Bioassay of toxic fescue hay fractions in cattle.

Progress: The source pasture for toxic tall fescue forage produced severe fescue foot in certain grazing cattle last year. This toxic forage will be extracted and purified fractions of this extract assayed for their toxicity in cattle. Forage, chemicals, equipment, cattle all are now in readiness. The extraction process has begun and following purification steps, the final fractions will be assayed February 1977.

20420-002

a. Specific Objective: Prepare and identify subfractions from extracts of toxic tall fescue hay.

Progress: Analysis of tall fescue by gas chromatography coupled with mass spectrometry revealed that summer-harvested tall fescue did not contain the hexose aldonic acid γ-lactone which has been present in toxic tall fescue forage harvested in the winter. High concentrations of quinic and shikimic acids were present in the summer harvested hay. The alkaloids of this summer-harvested forage were analyzed by GC-MS. One sample from a pasture which caused extreme heat intolerance and poor weight gains in cattle contained high concentrations of amide derivatives of loline, a pyrrolizidine alkaloid. Unsaturated pyrrolizidine alkaloids (known hepatotoxins) were not detected.

b. Specific Objective: Isolate, identify, and evaluate the effects of an antifungal antibiotic produced by Fusarium equiseti NRRL 6227 on fungi and small animals.

Progress: Fusarium acuminatum NRRL 6227 produces two mycotoxins, butenolide, and T-2 toxin, plus an antibiotic that causes incubating conidia and apical cells of 12 species of Penicillium to swell more than 5 diameters

while conidial germination and hyphal elongation is inhibited. Several other fungal genera are also affected by the antibiotic now characterized as a cyclic peptide or as a cyclodepsipeptide containing the amino acids glycine, glutamic acid, leucine, threonine, and tyrosine.

c. Specific Objective: Develop an assay and production procedure for moniliformin, a highly toxic mycotoxin produced by Fusarium moniliforme.

Progress: Fusarium moniliforme NRRL A2929 was identified as a producer of large amounts of the mycotoxin, moniliformin. A procedure for production, partial purification, and identification by TLC has been developed.

2. Plan of Work:

20420-001-A

Specific Objective: Assay anion fractions, subfractions, synthetic mixtures, or pure compounds for the ability to produce fescue foot.

Plan of Work: Determine the activity of test materials by IP cattle assay by a method previously developed.

20420-002

a. Specific Objective: Prepare anion fractions of toxic tall fescue and/or new varieties of tall fescue and further subfractionate one or more of such anion fractions.

Plan of Work: Extract chopped forage with aqueous ethanol, treat the extract with cation exchange resin, pass the treated extract through an anion exchange resin, and elute the absorbed anions with formic acid. Gradient elution will be used to obtain subfractions.

b. Specific Objective: Characterize both toxic and nontoxic anion fractions and subfractions.

Plan of Work: Initial separations and tentative assignments will be made by GC-MS and other forms of chromatography will be employed for isolating pure components. Tentative structures will be confirmed by IR, NMR, and other physical methods.

c. Specific Objective: A synthetic toxic anion fraction will be prepared from pure compounds.

Plan of Work: Pure compounds identified as being present in fractions which produce clinical signs of fescue foot in cattle will be combined in proper concentrations.

d. Specific Objective: The effect of amide derivatives of loline alkaloids on cattle gains will be studied.

Plan of Work: Assay the alkaloids of tall pastures by GC-MS. Obtain enough N-acetyl loline to determine its effect on animal gains.

RESEARCH ON CONSERVATION OF LAND AND MAINTENANCE OF ENVIRONMENTAL QUALITY

CRIS Work Units Listed by Work Reporting Unit

Work Reporting Unit (WRU)

Number	Title (Principal Investigator)
RECLAMATION AND REVEGETATION OF LAND AREAS DISTURBED BY MAN (p. 209)	
3102-20770-001	Trace element uptake and distribution in agricultural crops grown on disturbed lands (C. W. Blessin)

RECLAMATION AND REVEGETATION OF LAND AREAS DISTURBED BY MAN

A_1 Technological Objective: Utilize waste materials in reclaiming disturbed areas.

1. Progress Report (Narrative):

20770-001

a. Specific Objective: Conduct research on translocation of trace elements, including heavy metals, in food and feed crops grown on disturbed lands amended with sewage sludge.

Progress: Metal translocation studies for Zn, Mn, Cu, Pb, Cr, Cd, and Hg were conducted for corn plants grown on strip-mine soil amended with anaerobically digested sewage sludge. Differential metal accumulation rates in the seven plant tissues analyzed showed that generally the highest metal concentrations occur in the leaves and roots and the lowest in the grain and cob. With the exception of Mn and Hg, metal concentrations increased in tissues as a result of sludge application. The greatest increases were for Cd where concentrations in roots, lower stems, and leaves were, respectively, 59, 7.55, and 5.52 times greater than in counterpart tissues representing plants grown in untreated strip-mine soil. Plant metal availabilities for plant uptake were established for the soil samples adhering directly to the root system of the corn plants studied and these availabilities were compared to metal concentrations in the leaf and root tissues.

b. Specific Objective: Study potential new crops grown on stripmined land.

Progress: Crambe (Crambe abyssinica) and kenaf (Hibiscus cannabinus) were grown on stripmine land in three replications of four conditions. The variables were two levels of activated sludge, commercial fertilizer, and as is. Favorable response to sludge and fertilizer was obvious throughout the growing season. For crambe, control plots yielded fewer, less branched plants of lighter color, while fertilized plots produced more, bushier, deep green plants. Kenaf responded favorably to sludge and fertilizer throughout the growing season; however, the most obvious characteristic was color. Better germination of crambe in sludge-amended soil suggested that the sludge acted as a soil conditioner. Unfortunately, sludge harbors an abundance of weed seeds, and in spite of elevated herbicide applications, sludge treated crambe plots were extremely

weedy. Such plots were easily distinguishable by weed type and weed density. No crambe yield data was obtained because of weed problems and poor seed quality. Weeds were adequately controlled in the kenaf plots with the same herbicide. Yield of kenaf stems collected 1 month after a killing frost were, respectively, 4,500, 4,100, 3,800, and 2,700 pounds/acre from the tests: sludge II, sludge I, commercial fertilizer, and control. Soil and plant samples were collected from all plots for future heavy metal analyses.

2. Plan of Work:

20770-001

a. Specific Objective: Identify crops most suitable for growing in disturbed soils by investigating crop quality with respect to protein and ash content as well as uptake of heavy metals.

Plan of Work: Determine yield; compositional changes in protein and ash; and trace elements, including heavy metals, in selected above ground and root crops grown on mine waste and landfill amended with digested sewage sludge. The following crops: beans, beets, cabbage, carrots, cauliflower, eggplant, lettuce, onions, peas, peppers, radishes, spinach, sweet corn, tomatoes, and wheat will be examined. Analytical work on trace elements, especially lead, cadmium, mercury, and zinc will be concentrated on selected stored crops as time and quantity of crop permits.

b. Specific Objective: Repeat experiment of growing crambe and kenaf on stripmine land to broaden data base.

Plan of Work: The same plots will be planted to crambe and kenaf with identical fertilizer and sludge treatments used in 1976. Crambe will be drilled in 7-inch rows and herbicide type and/or amounts will be adjusted to better control weed problems originating primarily with the sludge. Kenaf herbicide treatment and planting density will be identical to 1976. Soil samples and plant materials will be collected for heavy metal analyses.

c. Specific Objective: Analyze soil and plant samples collected from 1976 trials.

Plan of Work: Samples will be prepared for analysis and analyzed for heavy metal content. Results will be evaluated in relation to plot treatment and anticipated end uses of the plant materials.

RESEARCH TO EXPAND AGRICULTURAL EXPORTS

CRIS Work Units Listed by Work Reporting Unit

Work Reporting Unit (WRU)

Number	Title (Principal Investigator)
TECHNOLOGIES AND PRODUCTS TO INCREASE EXPORTS OF AGRICULTURAL PRODUCTS (p. 213)	
3102-20650-001	Full-fat soy flours for use in foreign countries (G. C. Mustakas)
3102-20650-002	Basic engineering studies on preparation of soy foods for the export market (G. C. Mustakas and E. C. Baker)

TECHNOLOGIES AND PRODUCTS TO INCREASE EXPORTS OF AGRICULTURAL PRODUCTS

A. Technological Objective: Develop new and improved products suitable for the export market.

1. Progress Report (Narrative):

20650-001

a. Specific Objective: To improve flavor, lower processing cost, and utilize the whey fraction in producing the Lipid Protein Concentrate (LPC) product.

Progress: Soybean whey from the LPC process represents a major disposal problem and, unutilized, represents a loss of both protein and carbohydrate. Concentration of the whey from about 98% water to 15% water with significant BOD reduction has been achieved in a pilot plant reverse osmosis process. Cellulose acetate membranes in spiral wound modules were used. Solvent flux rates were studied as functions of temperature, pressure, and solids concentration of the feed. The capability of the membrane to concentrate solutions (solids retention) was independent of temperature but dependent on membrane porosity, pressure, and feed concentration. A mathematical model of the system has been found to fit the experimental data.

b. Specific Objective: To develop a high grade soy concentrate from defatted soy flours.

Progress: Two commercial firms are continuing their pilot scale production of low-cost soy beverage (LPC). During this period several batches of LPC curd were prepared and shipped to these companies for further processing and evaluation. In return, information from these companies was obtained particularly with respect to the spray-dried product.

The project begun last year as a result of technical inquiries from engineering and soybean processing firms to develop a simplified process for making high protein concentration by aqueous alcohol extraction is nearly completed. Bench scale experiments were carried out on defatted soybean flakes and flours extracted with aqueous alcohols to determine effects on flavor, yield, and functional properties. Generally, flavor scores were improved with alcohol mixtures having a higher level of water concentration although extraction yields were lower

due to the higher solubility losses of the carbohydrate component. When using azeotropic mixtures (higher alcohol composition), temperature improved flavor scores. Decreased particle size (flours vs flakes) resulted in slightly lower extraction yields but there was some evidence that better flavor was obtained as a result.

A contract with AID was completed in which an inventory of information on the utilization of soybeans as human food in developing nations, the state of the art of food uses of soybeans and the pattern of soybean consumption was compiled. Barriers affecting the use of soybeans as food were discussed and research needed to overcome such barriers was identified.

One of our engineers was invited by the American Soybean Association to Japan and Taiwan during May and June 1976 as part of their FY 76 marketing plan to describe in seminars and discussion sessions the latest technical information on processing and uses of soybeans for food utilization which included specific activities at NRRC on soybean utilization research.

2. Progress Reports (Achievements):

20650-001

Publications:

MUSTAKAS, G. C. AND V. E. SOHNS. Soy Processes, Equipment, Capital, and Processing Costs. Chapter in Farmers Cooperative Service Research Report No. 33, "Edible Soy Protein--Operational Aspects of Producing and Marketing." January 1976.

MUSTAKAS, G. C. Soy Beverages in World Feeding Programs. World Soybean Research. Proceedings of the World Soybean Research Conference, pp. 828-839. Interstate Printers Publishers, Inc. September 1976.

MUSTAKAS, G. C. Present Situation and Future Outlook of Soy Protein Foods. Soybean and Oil Processing (Taiwanese Journal) 7(1976):1-7.

Other Reports:

MUSTAKAS, G. C. Seminar program entitled "Present Situation and Future Outlook of Soy Protein Foods." Presented in Tokyo and Osaka, Japan, May 24-June 1, 1976, to representatives of

food industry and soy protein food manufacturers. Sponsored by American Soybean Association and Foreign Agricultural Service. Proceedings published in Japanese.

MUSTAKAS, G. C. Seminar program entitled "Present Situation and Future Outlook of Soy Protein Foods." Presented in Taipei and Hsinchu, Taiwan, June 1-5, 1976, to representatives of food industry and soy protein food manufacturers sponsored by American Soybean Association and Foreign Agricultural Service. Proceedings published in Chinese.

MUSTAKAS, G. C. "Soybean Technology and Whole Soy Emulsion Processing." Invited lecturer in the Meals for Millions Foundation International Short Course on Low-Cost High-Nutritious Foods. Santa Monica, California, October 18-20, 1976.

BAGLEY, E. B., C. W. HESSELTINE, G. C. MUSTAKAS, H. L. WANG, L. C. WANG, AND W. J. WOLF. Preliminary Report, "An Inventory of Information on the Utilization of Unprocessed and Simply Processed Soybeans as Human Food" submitted to AID. October 1976.

WANG, H. L., G. C. MUSTAKAS, W. J. WOLF, L. C. WANG, C. W. HESSELTINE, AND E. B. BAGLEY. "An Inventory of Information on the Utilization of Unprocessed and Simply Processed Soybeans as Human Food." Contract AID AG/TAB-225-12-76, Volume I and Volume II (Appendix on Recipes).

3. Plan of Work:

20650-002 (Replaced 20650-001 which terminated October 29, 1976)

a. Specific Objective: To investigate basic engineering and rheological aspects of extrusion in relation to developing new soy protein intermediates for use in foods in the export market.

Plan of Work: Examine fundamentals of extrusion of soybean products and blends to relate extrusion conditions (temperature, pressure, retention time, moisture content) and machine characteristics (screw and barrel design) to product properties. Emphasize basic theory and information needed for process scale-up calculations and for application to defatted and full-fat soy flours, protein isolates and concentrates, and to systems in which extrusion conditions are modified to control physical, organoleptic, and nutritive properties.

b. Specific Objective: To investigate the solvent extraction of soybeans in relation to product quality as a basis for expanded use in foods for the export market.

Plan of Work: Alcohol extracted samples from the cooperative project on high protein concentrates will undergo storage stability tests at 25°C to determine if a flavor reversion problem exists.

c. Specific Objective: Optimize the reverse osmosis process for soybean whey produced in the LPC process.

Plan of Work: The reverse osmosis treatment of LPC soy whey will be continued to study optimum conditions for the separation of water. Whey solids from the process will be isolated and their nutritional properties evaluated. The effects of operating conditions on costs, permeate quality and membrane life will also be determined.

d. Specific Objective: Process sprouted soybeans to produce soy flour and other soy products of reduced oligosaccharide concentration.

Plan of Work: Previous workers have found that a 1 or 2 day germination of soybeans lowers the levels of stachyose and raffinose, the two most important oligosaccharides related to flatulence in soybeans. No information on processing is available so a practical method is needed to integrate germination technology with an engineered process that will result in an improved quality soy product. New improved HP liquid chromatography techniques will be used to monitor the levels of oligosaccharides.

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